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EXPERIMENTAL STUDIES ON THE DURATION OF LIFE

I. INTRODUCTORY DISCUSSION OF THE DURATION OF LIFE IN DROSOPHILA[1]

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SUCH quantitative knowledge as exists of fundamental principles in the general biology of the duration of life has, in the main, been derived from an examination by purely statistical methods of human mortality records. Of course a good deal of information about the biology of death and duration of life of a general and non-quantitative character has been gained from experimental work on lower organisms. This literature has recently been reviewed by one of us (Pearl (1) to (7) inclusive). But the outstanding fact is that most of the existing *quantitative* data about duration of life are purely statistical, and derived from man as material.

The statistical method of acquiring knowledge of natural phenomena has a number of distinct and important limitations (cf. Pearl (8)). It is the settled policy of this department to check every conclusion drawn from purely statistical methods by an independent experimental investigation of the same problems, wherever in the nature of the case this is possible. Most problems of human vital statistics can not, in the nature of the case, be investigated experimentally, in any direct way with man himself as material. Probably this is chiefly the reason why all of the immense mass of data collected, and work done upon vital statistics has contributed so little in the

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way of general principles to the science of biology. In outlining the plans of the department at the time of its inauguration provision was made, as a major element in the whole organization scheme, for experimental work on the duration of life, to parallel as closely as possible, in respect of its problems and foci of interest, the statistical work of the department. The present paper is the first of a series which will appear dealing with the experimental side of our work.

Originally it was planned to use mice as the material for experimentation on the duration of life, and a large and flourishing colony was bred up in accordance with the most critical genetic standards for experimental material. Just as the colony was ready to start definitive experimentation with, an accident completely destroyed it.

It was then decided, after advising with a number of persons, notably Professor T. H. Morgan and Dr. Jacques Loeb, to take up *Drosophila* as material for the extensive program of experimental work which we had planned. This organism has the great advantage over any other which could be used, that its genetic behavior and potentialities are more thoroughly understood than those of any other animal, thanks to the epoch-marking researches of Morgan (9) and his students. It has the further great advantage that under certain conditions, which we now rather clearly understand, its duration of life, both in respect of means and of the l_x or d_x distributions of a life table, is extraordinarily like that of man, with one day in the life of the fly corresponding roughly to one year in the life of man.

The first paper in the series aims to present, as a background of reference for further contributions, the following essential items:

1. A brief review of what has been noted by previous workers regarding duration of life in *Drosophila,* and other insects in so far as the observations are quantitative in character.

2. The details of our material and methods of exper-

imentation, which are critically standardized and have been used in the work which will be described in subsequent papers.

3. The general form and characteristics of the mortality curves of *Drosophila,* presenting mortality tables for certain strains.

4. The influence of certain phases of the experimental technique employed upon the results.

Specific problems regarding the duration of life will be presented and discussed in the subsequent papers in the series.

Literature

The earliest mention we have found of observations on the duration of life of *Drosophila* is a casual reference in a paper by Moenkhaus (10) published in 1911 in which he makes the following statement in connection with egg counts: "We have kept females alive 153 days." There are no details of any kind, as to conditions or numbers involved.

The first paper to make more than casual reference to the duration of life of *Drosophila* is a paper published in 1913 by Hyde (11). In studying fertility and sterility in different strains of flies he found two strains which differed to a marked degree in respect of length of life, and made crosses to study the behavior of the shortened length of life of the mutant "truncate" in heredity. His numbers are small, but they show the characteristic increased vigor of F_1 hybrids. The shorter average age of the lumped F_2's indicates that there have segregated out in the F_2 generation some short-lived flies. His data, however, do not give, or allow us to get, separate averages for the truncate F_2's and the normal winged F_2's.

His data are summarized in Table I.

In 1914 Baumberger (12) published a paper in which he gives data on the length of life of different orders of insects without food as affected by different constant temperatures, and by exposure to two different temperatures. Since the insects were caught in a net as imagoes the total

longevity is not known, so that the results have little significance from the standpoint of exact studies. The 359 insects had at 72° F. an average longevity of 4.8 days with a maximum of 15 days, at 62° F. an average of 6 days with a maximum of 23, and at 42° F. an average of 10.9 days with a maximum of 39. For the second part of the experiment 184 larvæ of the oak-tree moth were used. The results are too conflicting to allow one to draw any definite conclusions.

TABLE I

HYDE'S DATA ON INHERITANCE OF DURATION OF LIFE IN DROSOPHILA

Type of Flies		In-bred Wild	Trun-cate	F_1	Truncate ♂ × Inbred ♀ F_2	Recip-ro-cal F_2	Total
No. of Flies.		191	272	42	128	89	722
Mean duration of life in days.	♂ and ♀	37.4	21.4	47.0	29.5	29.3	.....
	♂	40.5	26.9	47.8	32.8	31.1	.....
	♀	34.5	18.5	46.4	25.9	27.3	.....

In 1915 appeared Lutz's (13) paper on natural selection in which he finds in each sex a slight negative correlation between the length of adult life and the duration of the embryonic periods. The distributions which he has for normal length of adult life with varying temperature give the 250 ♂s an average duration of life of 36.3 days, and the 263 ♀s an average of 28.9 days. He also gives distributions in hours of duration of life of flies which were given water but no food, and the correlations of duration of life of these starved flies with wing measurements.

During 1916 and 1917 Loeb and Northrop (14–16) published a series of papers on the effects of food and temperature on duration of life in *Drosophila*. The first preliminary paper in 1916 gives the duration of life of cultures of *Drosophila* in water and in cane sugar at temperatures from 28° to 9° C., showing a temperature coefficient for the duration of life of about the order of magnitude of that of chemical reactions, namely of about 2 for a dif-

ference of 10° C. The averages were much lower than those found by Hyde and Lutz because, of course, of the inadequate food. At 19° C. the culture in water had an average duration of life of 4.1 days and those in 1 per cent. cane sugar of 12.3 days. In 1917 the experiments were repeated, using sterile flies on 2 per cent. glucose agar which was found to be a more adequate food. Similar results were obtained, getting a similar coefficient for the duration of the larval and pupa stages, and finding that the ratios of the duration of the three different stages remained approximately constant for the different temperatures. The averages here of the life of the imago are more of the order of those found by previous workers, 228 flies at 30° lived an average of 13.6 days, 70 flies at 25°, 28.5, and 49 flies at 20°, 40.2. Later in 1917 they published another paper in which they give 92.4 days as the average duration of life of 143 flies at 15°, and 120.5 days of 105 flies at 10° C., together with the frequency distributions from which the averages were obtained. They also present results with different food mixtures, and for the two sexes separately, finding that isolated males live a little longer than isolated females, or than the males when mixed with females.

In another paper in the same issue Northrop (17) gives the results of some experiments undertaken to determine the effect on the duration of life of the imago of prolonging the life of the larva by inadequate feeding (omitting yeast for different lengths of time). In this way the embryonic periods were prolonged from 8 to 17 days, but the duration of life of the adult remained the same in every case, ranging between 10.5 and 11.9 days at 27.5° C., at which temperature the four experiments, involving 644 flies, were performed.

In a recent paper Arendsen Hein (18) gives a few observations on duration of life in the meal-worm *Tenebrio molitor*. Thirty-two male beetles lived an average of 60 days, with a range from 39 to 113, and 32 females averaged 111 days, with a range from 89 to 132 days.

MATERIAL AND METHODS

The flies furnishing the data set forth in this paper belonged to five different basic laboratory stocks or strains of *Drosophila melanogaster*. Four of these stocks were obtained from Professor T. H. Morgan in December, 1919, and have been bred continuously in this laboratory since that time. The original individuals of the fifth stock were collected by one of us (R. P.) as wild flies at Eagle Point, Lake Memphremagog, Vermont, in the summer of 1920.

The stocks may be listed as follows:

1. *Old Falmouth.* Wild type fly, long bred in Morgan's laboratory. More inbred than 2.
2. *New Falmouth.* Wild type fly bred for about 6 months in Morgan's laboratory before we got our sample of it.
3. *Sepia.* A mutant stock carrying one third chromosome mutation, sepia eyes, in homozygous form. Other characters wild types. (Morgan.)
4. *Quintuple.* A synthetic stock, carrying five second chromosome mutations, each in homozygous form, as follows: Purple, arc, speck, vestigial, and black. Other characters wild type. (Morgan.)
5. *Eagle Point.* Wild type collected in summer of 1920, and since bred in this laboratory.

An account of the second chromosome mutations mentioned will be found in Bridges and Morgan (19) and Sturtevant (20). The discovery of the mutation sepia is noted by Muller (21).

The stocks are carried along in the laboratory in pure mass cultures in half-pint milk bottles. Those in an experiment on duration of life are tested relative to this character in one ounce shell vials.

The flies are all kept on a standard food mixture made up fresh each day, according to the following method:

For each 100 c.c. of water add 2 grams of agar-agar. Boil the agar and water until the agar is thoroughly dissolved. For each 100 c.c. of solution add 100 grams of ripe peeled mashed bananas. Boil five minutes. Pour into bottles which have been well heated in oven (or sterilized in autoclav). In the breeding bottles pour a layer 3/4 inch deep; in duration of life bottles a layer 1/2 inch deep. When

the food has partly cooled sprinkle on top of food the smallest possible amount of pulverized dry magic yeast (shaken from a can with one pin hole in cover). Put in breeding bottle a folded square of filter paper, and stopper with cotton batting.

The purpose of the filter paper is to furnish a dry place for the larvæ to crawl up and pupate on, and also to absorb some of the excess moisture which often forms on top of the food from the growing yeast. Filter paper has not been used in the small duration of life bottles, since no young are pupating there, and since it furnishes too many hiding places for the flies in the frequent transfers which have to be made in the duration of life tests. Excess of moisture on top of the food may become a source of error in duration of life experiments, because flies may drown in a small drop of water. Throughout our work we have been constantly on guard against this source of error and have tried a number of plans, with varying degrees of success to eliminate it entirely. Some of these experiments will be reported on in detail later on in this paper. In general it may be said here that this source of error from flies drowning need never be a significant one if due precautions are taken. We know that it has not been in our work.

Occasionally the yeast becomes too active at the edge of the food and causes the whole food mass to rise in the bottle. In an attempt to eliminate this accident yeast in dilute solutions was added to the boiled bananas and agar and was sprayed on top of the food. The results were not particularly favorable. The pulverized dry yeast added in the most minute quantity possible is the most satisfactory standard method yet found. We expect to continue attempting to get the food conditions more and more nearly ideal and identical in every experiment, but we feel reasonably certain that in all of the experiments we shall report, even including the very first in point of time, the precautions taken to standardize food and to guard against accidental death were sufficient to insure statistical accuracy in the results. Whatever environ-

mental differences in respect of food have existed in our experiments have been randomly distributed among the different groups in any given experiment. We have had very little trouble at any time with moulds in the cultures, the frequent transfers in the duration of life bottles in an experiment preventing them from getting any start.

The stock bottles holding reserve stocks of flies have been kept at the varying temperature of the room, but all experimental flies have been kept in electric incubators at 25° C., in which recording thermometers have been placed to insure that no fluctuations of temperature have occurred without our knowledge. All the experiments on duration of life and their results recorded in this paper have been carried through at the constant temperature of 25° C. We have settled on this as a normal for this particular element of the environmental complex.

During the first year of the experimental work no attempt was made to keep the different generations separate in the stock bottles, the process being merely to keep enough bottles (generally 4) of each stock to insure always having pupæ and newly emerging flies on hand for any matings and experiments to be started. Each week all the flies old and young together from the oldest bottle of each stock were transferred to a fresh bottle. In this way each bottle was kept 4 weeks and there were always on hand bottles with flies in all stages of development.

In January, 1921, it was decided that it would be desirable to keep the generations separate in the stock bottles. All stock bottles were emptied on January 11, and flies in the stock bottles on January 14 were arbitrarily called generation 0. From that time on the procedure has been to empty out all the parent flies from each stock bottle 7 days after the bottle was started (when there are usually a large number of larvæ and some pupæ formed). The bottles are then left for 7 days longer, during which time enough flies emerge to start a fresh bottle for the next generation. Several bottles are kept of each stock, as

before, to insure always having on hand newly emerging flies with which to start experiments.

When any experiment is to be started flies are taken from the stock bottles and etherized within 4 hours of emerging (usually sooner), before the wings have unfolded, so that they are surely virgin. Matings are made up as desired, putting the mated flies in half-pint milk bottles in the incubator. The parents are taken from the mating bottles in 8 or 9 days (before any young begin to emerge), and removed to a second mating bottle if a larger sample of progeny is desired than can be obtained from one bottle. As the offspring begin emerging they are shaken out every morning from the mating bottle to a small shell vial. Thus all the flies in a small bottle are the same age, and are properly labelled with mating number and date of emergence. Then every morning all these small bottles are looked over and those with dead flies separated out. After all have been looked over, the live flies in the bottles which have dead ones are shaken across to fresh bottles, the dead flies taken out and sexed, and all the pertinent facts as to duration of life, etc., recorded on printed blanks, from which the records are later (when all the flies of an experiment have died off) coded, punched on Hollerith cards, and sorted and tabulated by Tabulating Machine Company electric sorting and tabulating machines. Flies from any small bottles which are not changed (because of dead flies) within five days of the last previous transfer are transferred on the fifth day to fresh food. The physical manipulation is too great with the numbers we desire to use to admit of changing all the bottles every day, which would be the ideal way to keep food conditions absolutely identical for all the flies. Changing every five days keeps them approximately so.

We desire to record our indebtedness to Mr. James Krucky, technical assistant in this work, for his painstaking care and fidelity to the highest ideals of exact experimental work.

In the work discussed in this paper no attempt has

been made to keep the flies in aseptic culture as had been done by Loeb and Northrop (*eve. cit.*) and other workers. Our choice in the matter has not been dictated by technical difficulties, which are not great, but has been deliberate. Aseptic life is by no means normal life for *Drosophila.* Normally it is as loaded with a bacterial flora as we are. It was felt that in the beginning it would be well to establish norms of duration of life for normal life conditions. Later we expect to make a special study of duration of life under aseptic conditions.

Duration of life in this work with *Drosophila* is always measured in days, and all of our records relate to duration of adult or imaginal life. No account is taken in any figures of the larval or pupal stages. The reason for this convention is first accuracy and second convenience. It is far more difficult to measure accurately either larval or pupal duration of life than it is imaginal. And from the point of view of these studies nothing significant in principle is lost by dropping these early stages, so far as we have been able to discover, either from the literature or experience with the flies.

MORTALITY CURVES

The most exact and comprehensive manner in which the facts about the duration of life in any organism can be presented is by means of life tables, of the type used for many years by actuaries in their work. The biologically essential features of a life table may be mentioned here briefly for the benefit of biologists not immediately familiar with the development of actuarial science. A complete life table includes, *inter alia,* the following items:

1. The number of individuals surviving up to each of the ages x_0, x_1, etc., out of a given number (1,000 or 10,000 or whatever number one chooses) assumed to have started life together at exactly the same instant of time. These survival frequencies taken together constitute what is technically known as the l_x line of a life table.

2. The number of individuals dying within any short

interval of time, say between x and $x+1$, or $d_x = l_x - l_{x+1}$. These frequencies of death taken together constitute the so-called d_x line of a life table.

3. The death rates at each time (or age) x; *i.e.*, the ratio of the deaths between time x and $x+1$ to the survivors at the time x. These observations together constitute what is known as the q_x line of a life table $q_x = (l_x - l_{x+1})/l_x$.

4. The curtate expectation of life of individuals at a given age x. This is the mean or average after life time of all those individuals alive at age x neglecting fractions of the x interval. These observations together constitute the e_x line of a life table.

$$e_x = \frac{l_{x+1} + l_{x+2} + l_{x+3} + \text{etc.}}{l_x}.$$

These simple definitions state with entirely sufficient accuracy for present purposes the significance of the constants which we shall present. Any one wishing to go more particularly into details of actuarial methods will find a useful elementary introduction in Henderson (22) or Dawson (23).

It is our purpose to present here life tables for four groups of flies, to serve, first, to show the general laws of mortality in *Drosophila* as compared with man, the only other organism for which we have extensive and exact life tables; and second, as a normal base for comparison in experimental work on *Drosophila* to be reported in subsequent papers.

The groups for which complete tables are presented are these:

1. *Long-winged males.* This table includes all our data up to June, 1921, on normal (*i.e.*, not experimentally modified) duration of life of male *Drosphila* individuals at 25° C. belonging to the following stocks (cf. p. 486 *supra*): Old Falmouth, New Falmouth, Sepia and Eagle Point. In these stocks all the individuals have wild type wings, hence the designation "long-winged."

2. *Long-winged females.* The corresponding table to 1, but for females.

3. *Short-winged males.* This table includes all our data up to June, 1921, on the normal duration of life at 25° C. of males belonging to the Quintuple stock. These flies carry the wing mutation vestigial; hence the designation "short-winged."

4. *Short-winged females.* The corresponding table to 3, but for females.

We have tried a number of different plans for the graduation of these tables, and wish to acknowledge gratefully the helpful suggestions of our colleague in the department, Dr. Lowell J. Reed, in connection with this phase of the work. It was first found that a rather satisfactory result could be obtained by fitting a logarithmic parabola of the type

$$y = a + bx + cx^2 + d \log x$$

to the q_x data. Working from this as a basis we finally decided that, as a practical matter, results on the whole most satisfactory could be got by the following type of graduation.

$$\log l_x = e^{\mathfrak{a}\, x}(a + bx + cx^2 + dx^3). \qquad \text{(i)}$$

This amounts to asserting that the instantaneous death rate increases with age as a modified logarithmic function of x.

The actual equations for the four calculated l_x lines of Tables II to V inclusive, together with the absolute number of individuals on which the curves are based, are as follows:

Long winged ♂*s* (4,586 flies):

$$\log l_x = e^{-.00853509x}(3.0041905 - .02937911x + .000140245x^2 - .0000015897x^3). \qquad \text{(ii)}$$

Long winged ♀*s* (5,426 flies):

$$\log l_x = e^{-.00481563x}(3.0042303 - .01869993x + .000059620x^2 - .0000020438x^3). \qquad \text{(iii)}$$

Short winged ♂*s* (854 flies):

$$\log l_x = e^{-.0571867x}(3.0085931 - .17931770x + .004010630x^2 - .0000332501x^3). \qquad \text{(iv)}$$

Short winged ♀*s* (906 flies):

$$\log l_x = e^{-.0460428x}(3.0116555 - .14948615x + .002851219x^2 - .0000210642x^3). \qquad \text{(v)}$$

The plan of arrangement of Tables II to V is as follows: The first column gives the age of the flies in days, starting theoretically from the time of the emergence of the imago from the pupa as zero. Since the flies spend on the average a day in the breeding bottle before they are taken out into the small duration-of-life bottles, and the deaths are not observed for this interval, our distributions as recorded actually start with age 1 instead of age 0. The next two columns give the *observed* deaths and survivors on the basis of a thousand individuals at "birth" (here emergence as imago). The next three columns give the *calculated* (graduated) values deduced from equations (ii) to (v) above; first the l_x line, next the q_x, and finally the e_x, the latter values being of course in days. Owing to the fact that no premium rates are likely to be calculated from these life tables, we have not thought it necessary to keep but one place of decimals in the case of the q_x and e_x lines, and none whatever in the l_x line. Of course, in the computations more decimal places were kept, and these life tables may be regarded as accurate to a considerably higher degree than the figures as here published indicate. But, on the other hand, so far as we can see, the figues here tabled are sufficiently detailed for any use to which they are ever likely to be put.

The l_x lines of Tables II to V are shown graphically in Figs. 1 and 2. The diagrams are plotted to an arithlog grid, the scale of the abscissæ being divided arithmetically, and that of the ordinates logarithmically. Field (24) has shown the advantages of this method of plotting life table l_x lines. He says:

> In the natural-scale diagram the descent of the curve expresses the number of deaths in a year among the survivors to a given age. This is not the usual way of stating death-rates; nor is it a convenient method, since the absolute number of deaths is a joint resultant of two factors which might better be considered separately—the probability of death at the specified age, and the number of persons at that age and subject to that hazard. We are ordinarily more concerned with the probability alone, or, which is much the same

TABLE II

LIFE TABLE FOR DROSOPHILA—LONG-WINGED MALES

Age in Days	Observed		Calculated			Age in Days	Observed		Calculated		
	d_x	l_x	l_x	q_x	e_x		d_x	l_x	l_x	q_x	e_x
1.....	5	1,000	1,000	9.6	41.0	50.....	15	363	368	45.8	14.2
2.....	12	995	990	9.7	40.4	51.....	20	348	351	47.7	13.8
3.....	6	983	981	9.7	39.7	52.....	19	328	334	49.6	13.5
4.....	13	977	971	9.7	39.1	53.....	22	309	318	51.6	13.1
5.....	10	964	962	9.9	38.5	54.....	16	287	301	53.7	12.8
6.....	10	954	952	10.0	37.9	55.....	13	271	285	55.7	12.4
7.....	15	944	943	10.1	37.2	56.....	19	258	269	57.9	12.1
8.....	9	929	933	10.3	36.6	57.....	12	239	254	60.2	11.8
9.....	9	920	924	10.4	36.0	58.....	19	227	238	62.5	11.5
10.....	9	911	914	10.6	35.4	59.....	13	208	224	64.8	11.2
11.....	12	902	904	10.8	34.7	60.....	12	195	209	67.3	10.9
12.....	8	890	895	11.0	34.1	61.....	18	183	195	69.8	10.6
13.....	8	882	885	11.3	33.5	62.....	8	165	181	72.4	10.3
14.....	11	874	875	11.6	32.8	63.....	13	157	168	75.2	10.1
15.....	8	863	865	12.0	32.2	64.....	12	144	156	77.9	9.8
16.....	14	855	854	12.2	31.6	65.....	13	132	143	80.8	9.5
17.....	8	841	844	12.6	31.0	66.....	14	119	132	83.6	9.3
18.....	13	833	833	13.0	30.3	67.....	7	105	121	86.7	9.0
19.....	10	820	822	13.4	29.7	68.....	8	98	110	89.8	8.8
20.....	11	810	811	13.9	29.1	69.....	5	90	100	92.9	8.6
21.....	16	799	800	14.4	28.5	70.....	8	85	91	96.1	8.4
22.....	6	783	789	14.9	27.9	71.....	5	77	82	99.6	8.1
23.....	13	777	777	15.4	27.3	72.....	7	72	74	102.9	7.9
24.....	11	764	765	16.0	26.7	73.....	8	65	67	106.4	7.7
25.....	11	753	753	16.6	26.2	74.....	3	57	59	110.0	7.5
26.....	10	742	740	17.3	25.6	75.....	9	54	53	113.8	7.3
27.....	10	732	727	17.9	25.0	76.....	2	45	47	117.3	7.1
28.....	14	722	714	18.7	24.4	77.....	8	43	41	121.5	6.9
29.....	11	708	701	19.4	23.9	78.....	4	35	36	125.4	6.8
30.....	15	697	687	20.2	23.3	79.....	4	31	32	129.2	6.6
31.....	13	682	673	21.1	22.8	80.....	3	27	28	133.6	6.4
32.....	11	669	659	21.9	22.3	81.....	3	24	24	137.5	6.3
33.....	15	658	645	22.9	21.8	82.....	4	21	21	142.0	6.1
34.....	7	643	630	23.8	21.2	83.....	2	17	18	146.4	5.9
35.....	18	636	615	24.8	20.7	84.....	1	15	15	151.0	5.8
36.....	15	618	600	25.8	20.2	85.....	2	14	13	156.2	5.7
37.....	19	603	584	26.9	19.7	86.....	2	12	11	160.2	5.5
38.....	13	584	569	28.1	19.3	87.....	1	10	9	164.5	5.4
39.....	22	571	553	29.3	18.8	88.....	2	9	8	170.6	5.2
40.....	15	549	536	30.5	18.3	89.....	2	7	6	175.6	5.1
41.....	13	534	520	31.8	17.9	90.....	0	5	5	180.4	5.0
42.....	23	521	503	33.2	17.4	91.....	1	5	4	185.0	4.9
43.....	19	498	487	34.5	17.0	92.....	1	4	3	189.7	4.8
44.....	22	479	470	36.0	16.6	93.....	1	3	3	196.3	4.6
45.....	18	457	453	37.5	16.1	94.....	0	2	2	201.8	4.5
46.....	22	439	436	39.0	15.7	95.....	1	2	2	207.5	4.4
47.....	19	417	419	40.7	15.3	96.....	0	1	1	212.8	4.3
48.....	15	398	402	42.3	14.9	97.....	0	1	1	218.3	4.2
49.....	20	383	385	44.0	14.6						

TABLE III

LIFE TABLE FOR DROSOPHILA—LONG-WINGED FEMALES

Age in Days	Observed		Calculated			Age in Days	Observed		Calculated		
	d_x	l_x	l_x	q_x	e_x		d_x	l_x	l_x	q_x	e_x
1.....	5	1,000	1,000	9.7	38.8	46.....	19	386	384	46.4	14.3
2.....	14	995	990	9.7	38.1	47.....	19	367	367	48.3	13.9
3.....	10	981	981	9.8	37.5	48.....	13	348	349	50.2	13.6
4.....	13	971	971	9.9	36.9	49.....	23	335	331	52.1	13.2
5.....	12	958	961	10.0	36.2	50.....	16	312	314	54.1	12.9
6.....	10	946	952	10.1	35.6	51.....	20	296	297	56.1	12.6
7.....	13	936	942	10.3	34.9	52.....	15	276	281	58.2	12.3
8.....	8	923	932	10.5	34.3	53.....	15	261	264	60.4	12.0
9.....	11	915	923	10.8	33.6	54.....	17	246	248	62.6	11.7
10.....	13	904	913	11.0	33.0	55.....	11	229	233	64.9	11.4
11.....	12	891	903	11.4	32.4	56.....	17	218	218	67.2	11.1
12.....	6	879	892	11.8	31.7	57.....	13	201	203	69.6	10.8
13.....	9	873	882	12.1	31.1	58.....	12	188	189	72.0	10.5
14.....	12	864	871	12.4	30.4	59.....	15	176	175	74.5	10.3
15.....	8	852	861	12.9	29.8	60.....	12	161	162	77.1	10.0
16.....	12	844	849	13.3	29.2	61.....	11	149	150	79.8	9.8
17.....	9	832	838	13.9	28.6	62.....	11	138	138	82.4	9.6
18.....	11	823	827	14.4	28.0	63.....	9	127	126	85.1	9.3
19.....	17	812	815	15.0	27.4	64.....	9	118	116	87.9	9.1
20.....	10	795	802	15.6	26.8	65.....	12	109	105	90.8	8.9
21.....	22	785	790	16.2	26.2	66.....	10	97	96	93.8	8.7
22.....	10	763	777	16.9	25.6	67.....	7	87	87	96.7	8.5
23.....	16	753	764	17.6	25.0	68.....	11	80	78	99.8	8.3
24.....	14	737	751	18.4	24.4	69.....	5	69	71	102.9	8.1
25.....	10	723	737	19.2	23.9	70.....	10	64	63	106.2	7.9
26.....	12	713	723	20.1	23.3	71.....	3	54	57	109.2	7.7
27.....	14	701	708	20.9	22.8	72.....	7	51	50	112.5	7.5
28.....	18	687	693	21.9	22.2	73.....	3	44	45	115.9	7.3
29.....	16	669	678	22.9	21.7	74.....	4	41	40	119.4	7.2
30.....	16	653	663	23.9	21.2	75.....	5	37	35	123.0	7.0
31.....	12	637	647	24.9	20.7	76.....	1	32	31	126.2	6.8
32.....	13	625	631	26.0	20.2	77.....	7	31	27	130.2	6.7
33.....	15	612	614	27.2	19.7	78.....	4	24	23	133.4	6.5
34.....	14	597	597	28.4	19.2	79.....	1	20	20	137.5	6.4
35.....	18	583	581	29.6	18.8	80.....	3	19	17	141.6	6.2
36.....	17	565	563	30.9	18.3	81.....	2	16	15	144.9	6.1
37.....	16	548	546	32.2	17.9	82.....	4	14	13	149.0	6.0
38.....	17	532	528	33.6	17.4	83.....	1	10	11	153.0	5.8
39.....	19	515	511	35.0	17.0	84.....	1	9	9	157.8	5.7
40.....	17	496	493	36.5	16.6	85.....	1	8	8	161.5	5.6
41.....	15	479	475	38.0	16.2	86.....	2	7	6	164.9	5.4
42.....	13	464	457	39.7	15.8	87.....	0	5	5	169.7	5.3
43.....	15	441	439	41.2	15.4	88.....	1	5	4	173.3	5.2
44.....	21	426	420	42.9	15.0	89.....	1	4	4	180.1	5.1
45.....	19	405	402	44.6	14.6	90.....	0	3	3	183.6	5.0
						91.....	1	3	2	188.7	4.9
						92.....	0	2	2	192.1	4.8
						93.....	1	2	2	195.1	4.7
						94.....	0	1	1	204.5	4.6
						95.....	0	0	1	209.5	4.5

TABLE IV

LIFE TABLE FOR DROSOPHILA—SHORT-WINGED MALES

Age in Days	Observed		Calculated			Age in Days	Observed		Calculated		
	d_x	l_x	l_x	q_x	e_x		d_x	l_x	l_x	q_x	e_x
1.....	6	1,000	1,000	25.6	14.2	24.....	16	151	155	107.5	8.0
2.....	27	994	974	31.5	13.6	25.....	13	135	139	107.3	7.8
3.....	30	967	944	37.5	13.0	26.....	11	122	124	107.1	7.7
4.....	34	937	908	43.3	12.4	27.....	6	111	111	107.2	7.5
5.....	38	903	869	48.9	12.0	28.....	20	105	99	107.3	7.2
6.....	36	865	826	54.6	11.5	29.....	8	85	88	107.9	7.0
7.....	85	829	781	60.0	11.1	30.....	7	77	79	109.2	6.7
8.....	66	744	734	65.5	10.8	31.....	13	70	70	111.1	6.4
9.....	55	678	686	70.4	10.5	32.....	7	57	62	114.5	6.1
10.....	52	623	638	75.2	10.2	33.....	9	50	55	119.3	5.8
11.....	44	571	590	79.8	9.9	34.....	5	41	49	126.2	5.4
12.....	48	527	543	84.0	9.7	35.....	4	36	42	135.4	5.0
13.....	21	479	497	87.9	9.5	36.....	6	32	37	147.4	4.7
14.....	49	458	454	91.5	9.3	37.....	4	26	31	162.7	4.3
15.....	53	409	412	94.8	9.1	38.....	1	22	26	182.2	3.9
16.....	43	356	373	97.7	9.0	39.....	6	21	21	206.2	3.6
17.....	24	313	337	100.2	8.9	40.....	2	15	17	234.7	3.3
18.....	28	289	303	102.1	8.7	41.....	4	13	13	268.6	3.0
19.....	22	261	272	104.1	8.6	42.....	1	9	10	308.0	2.7
20.....	19	239	244	105.3	8.5	43.....	2	8	7	352.9	2.4
21.....	24	220	218	106.3	8.4	44.....	5	6	4	403.0	2.2
22.....	17	196	195	106.8	8.3	45.....	0	1	3	457.9	2.0
23.....	28	179	174	107.2	8.1	46.....	0	1	1	516.0	1.8

thing, with the proportion of those persons of given age who die in the course of a year. Precisely this relative mortality rate determines the slope of the curve in the logarithmic figure, for here, as always, a given distance on the logarithmic scale denotes a certain proportion of change. Hence the more steeply the logarithmic curve descends, the higher is the relative mortality which it indicates. Hence, too, it is possible to provide a key to the diagram in the form of standard sample slopes and corresponding numerical death-rates, which hold true for all parts of the curve.

From these tables and the diagrams, the following points are to be noted:

1. It is obvious that the laws of mortality are fundamentally similar in *Drosophila* to what they are in man, with the one striking and outstanding difference that since in the case of the *Drosophila* life tables we are deal-

TABLE V

LIFE TABLE FOR DROSOPHILA—SHORT-WINGED FEMALES

Age in Days	Observed		Calculated			Age in Days	Observed		Calculated		
	d_x	l_x	l_x	q_x	e_x		d_x	l_x	l_x	q_x	e_x
1	10	1,000	1,000	30.2	15.8	27	13	177	165	93.1	8.5
2	26	990	970	33.9	15.2	28	18	164	149	94.6	8.3
3	37	964	937	37.6	14.7	29	11	146	135	96.1	8.0
4	31	927	902	41.2	14.3	30	20	135	122	98.1	7.8
5	47	896	865	44.7	13.9	31	15	115	110	100.2	7.5
6	27	849	826	48.1	13.5	32	15	100	99	102.8	7.2
7	50	822	786	51.5	13.1	33	8	85	89	106.3	6.9
8	78	772	746	54.7	12.7	34	5	77	80	110.0	6.6
9	45	694	705	57.9	12.4	35	7	72	71	114.5	6.3
10	63	649	664	60.9	12.1	36	10	65	63	120.2	6.0
11	34	586	624	63.8	11.8	37	6	55	55	127.1	5.7
12	35	552	584	66.6	11.6	38	7	49	48	134.8	5.4
13	25	517	545	69.2	11.3	39	8	42	42	144.4	5.1
14	39	492	507	71.7	11.1	40	8	34	36	155.8	4.8
15	31	443	471	74.1	10.9	41	8	26	30	168.4	4.5
16	30	412	436	76.2	10.7	42	3	18	25	183.8	4.2
17	28	382	403	78.3	10.5	43	1	15	20	201.3	3.9
18	28	354	371	80.2	10.3	44	3	14	16	221.2	3.6
19	25	326	341	82.0	10.1	45	1	11	13	243.8	3.3
20	14	301	313	83.6	9.9	46	0	10	10	269.9	3.1
21	20	287	287	85.1	9.7	47	2	10	7	297.0	2.8
22	14	267	263	86.6	9.5	48	0	8	5	328.9	2.6
23	21	253	240	87.9	9.3	49	2	8	3	363.3	2.4
24	21	232	219	89.2	9.1	50	3	6	2	401.0	2.2
25	16	211	199	90.4	8.9	51	1	3	1	457.2	2.0
26	18	195	181	91.7	8.7						

ing only with the duration of imaginal life, the important infant and early childhood mortality component of the human d_x line is entirely omitted. With this difference in mind, it is apparent that the remainder of the l_x curve for the flies is essentially similar to any human l_x curve. Further on, we shall make a more detailed comparison between *Drosophila* and human curves.

2. There is evidently a fundamental and marked difference between the long-winged and short-winged groups in respect of the duration of life. This difference is somewhat more marked in the case of the males (Fig. 1) than in the females, but it is sufficiently definite and clear-cut

FIG. 1. Diagram showing the observed and graduated l_x points for long-winged and short-winged males, respectively. The small circles are the observations and the smooth lines the fitted curves from equations (ii) and (iv). In order not to overcrowd the diagram, only every second observation is shown.

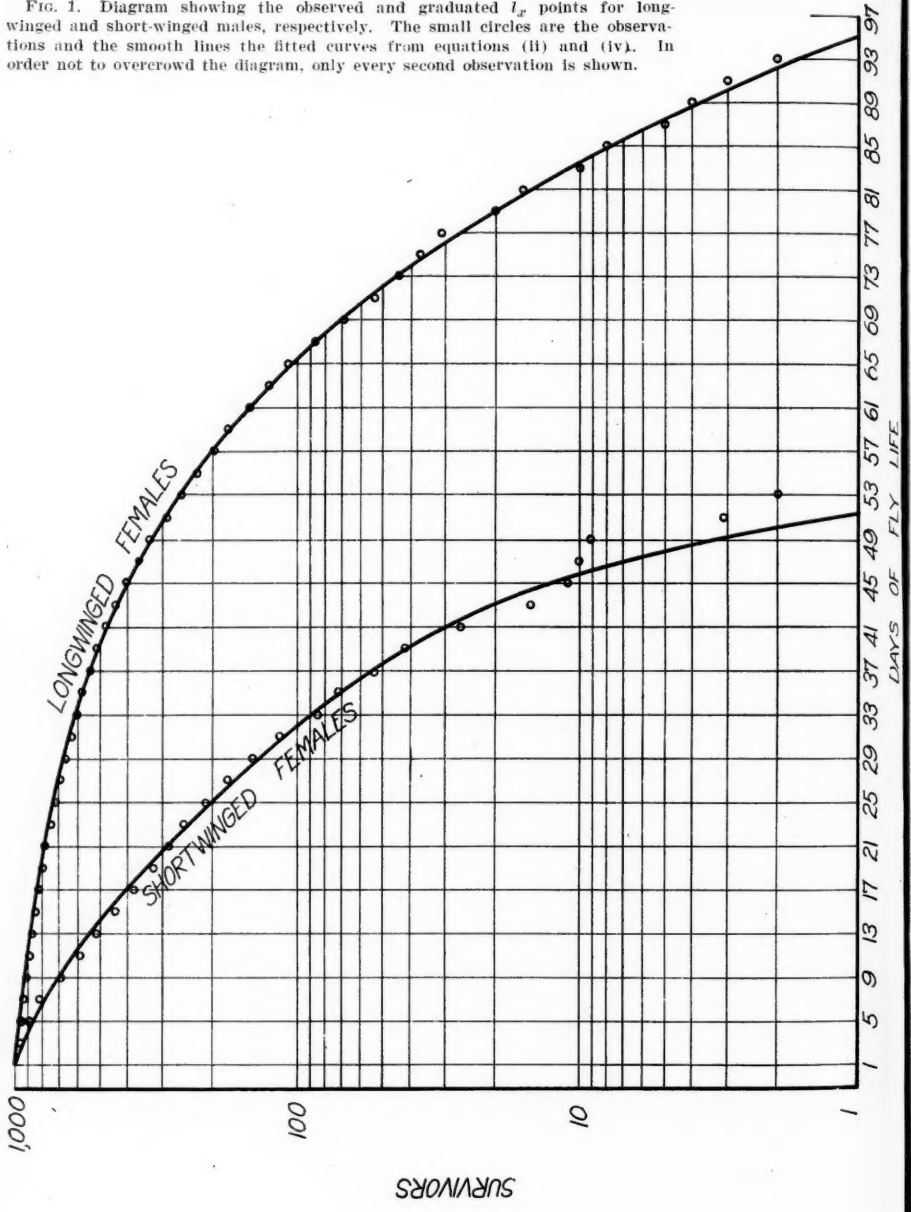
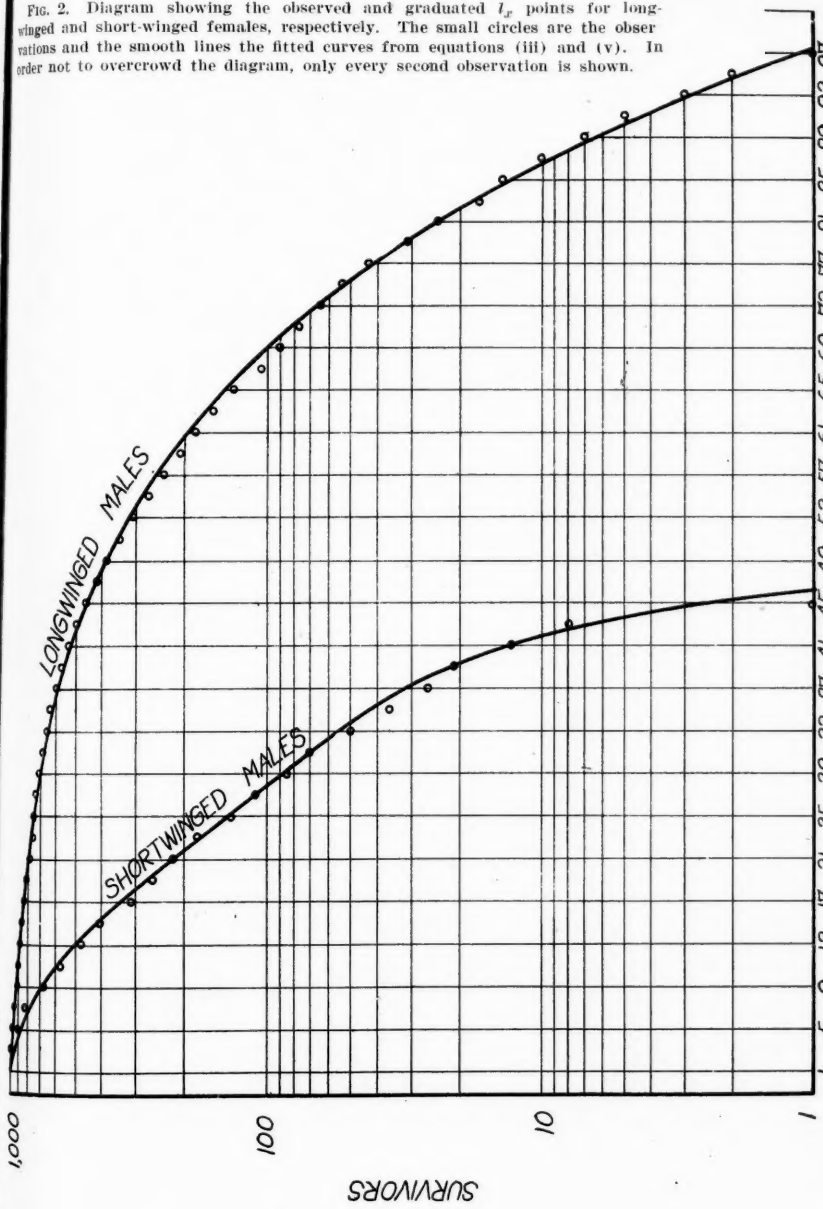


FIG. 2. Diagram showing the observed and graduated l_x points for long-winged and short-winged females, respectively. The small circles are the observations and the smooth lines the fitted curves from equations (iii) and (v). In order not to overcrowd the diagram, only every second observation is shown.



in both cases. Broadly speaking, the wild type long-winged flies have from two to three times as great an expectation of life at any age as do the flies of the Quintuple stock. Since all of these flies lived under substantially identical environmental conditions, as has been set forth earlier, it follows that the basis of the great difference in expectation of life between these two groups, as exemplified in Figs. 1 and 2, *is hereditary and not environmental.*

3. It is apparent that on the whole the graduations given by equations of the type of (i) are very satisfactory, and as good as could reasonably be expected on experience bases of the magnitude of those here dealt with. Undoubtedly the curves would be slightly more smooth if we had larger experience, especially in Tables IV and V, where we are dealing with less than a thousand flies in each case, but in the main the curves fit the observations very well.

4. The death rates (q_x) generally increase steadily with advancing age. An exception to this rule is the slight dip between ages 25–28 in the short-winged ♂ table.

In Figs. 3 and 4 the *Drosophila* l_x lines are compared with the human l_x line taken from Glover's (25) 1910 U. S. Life Tables. In order to make a just comparison, the human l_x line is displaced to the left in the diagrams until age fifteen of human life coincides with age one of the fly curve. This drops out the infant and childhood mortality component of the human curve. It will be understood that in the present instance, this is a somewhat arbitrary and purely graphic procedure. Whether the point which *exactly* corresponds on the human curve to the beginning of the *Drosophila* imago curve is exactly 15 years or 13 or 14, or some other near-by value, is a matter for further research. In a broad way, however, it is clear that the two lines must be taken to correspond at something like this point in the human curve.

From these diagrams, it is apparent that, after leaving out the infant mortality component of the human curve,

the essential difference between the human and *Drosophila* l_x curves is that, up to what may be designated as the end of the middle life portion (and even into the early part of the old age portion), human beings have a *relatively* better expectation of life than does *Drosophila*. On the other hand, in the extreme old age portion of the curve, the *Drosophila* expectation of life is relatively

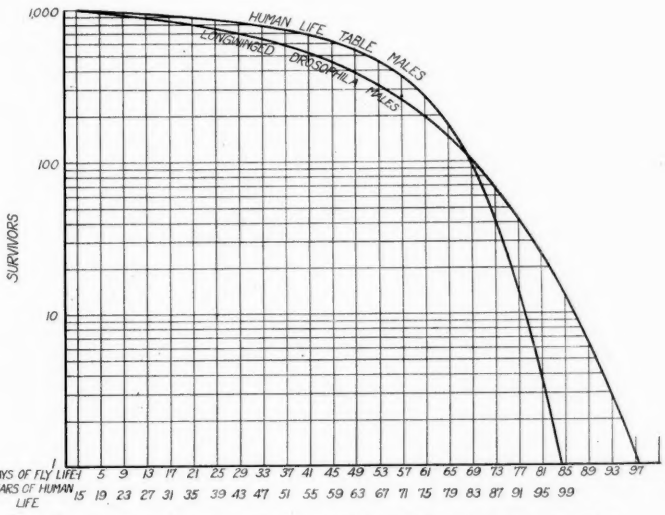


FIG. 3. A comparison of human l_x and *Drosophila* l_x lines for males.

better than the human. The result then is as though some external power had seized the *Drosophila* l_x line at about the middle of its course and bent it to a sharper angle in that region, stretching it at that point upward and to the right and by this process converted it into the human curve. Suppose one of the *Drosophila* l_x lines, as shown in Fig 3, to be a thin, flexible whalebone rod, possessing mass. Then move a point on that rod standing say just above the final *A* in "*Drosophila*" in Fig. 3, up to a point where it exactly coincides with the human life table curve. Then the whole of the rest of the *Drosophila* curve would fall into about the same posi-

tion as is occupied now by the human life table curve. Put in another way, what appears to have happened is that, as compared with *Drosophila,* more human beings are able to live through middle life, but at the expense of those who, if the mortality law was the same as in *Drosophila,* would live to extremely advanced old ages. As a matter purely of speculation in the present stage of our knowledge, it may be suggested that the *Drosophila*

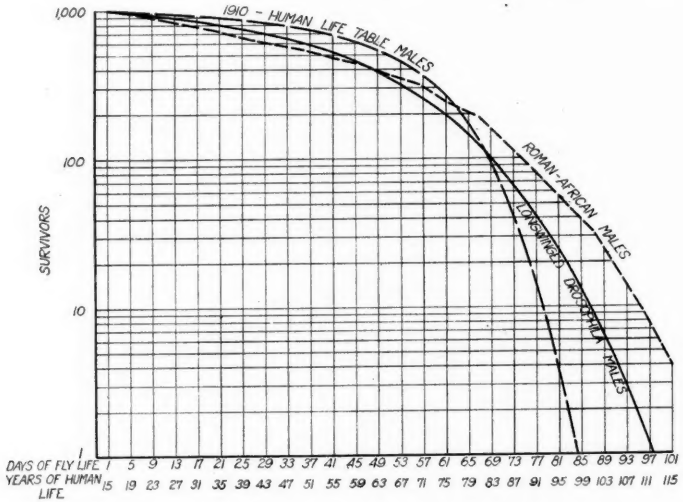


FIG. 4. A comparison of human l_x and *Drosophila* l_x lines for females.

l_x curves represent more nearly the normal, fundamental, biological law of mortality, and that the human curve has been warped from this form as a result of those activities which may be comprised under the terms public health and sanitation. It is to be understood that at present we offer this merely as a suggestion and in no way as a settled conclusion. It is, however, clear that the effect which we should expect these activities to have upon the form of the l_x line is exactly of the sort which makes the human curve different from the *Drosophila* in fact.

In this connection Fig. 5 is of interest and significance.

It compares, for males, two human l_x lines nearly 2,000 years apart in point of time, with the long-winged *Drosophila* l_x line of Table II. The two human lines are (*a*) Glover's, 1910 U. S. table (as in Fig. 3), and (*b*) Macdonell's (26) observed l_x line from the population of Roman provinces in Africa at about the beginning of the Christian era, his data having been taken from gravestone inscriptions. We calculated the l_x line here plotted from Macdonell's tabled d_x data, determining an l_x point at each quinquennium. This smooths the Roman-African figures somewhat, and makes the l_x line so determined lie very slightly higher all along its course than would be the case if we used a more elaborate and exact mathematical procedure. The error, however, is so small that it would scarcely be discernible in the scale at which Fig. 5 is reproduced.

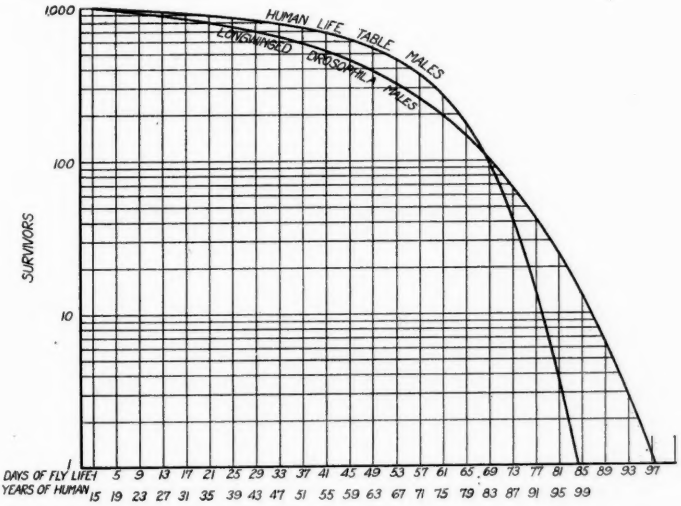


FIG. 5. A comparison of *Drosophila* mortality with human mortality at two periods (*a*) early in the Christian era, and (*b*) in 1910.

It is at once apparent from Fig. 5 that the *Drosophila* survival curve runs, in general throughout its course, *between* the curve for human beings 18 or 19 centuries

ago and that for the present time. As compared with Glover's 1910 U. S. tables, the Roman population of Africa at the dawn of our era was, in respect of the course of its mortality, even more *Drosophila*-like than *Drosophila* itself! Now in Roman Africa there was relatively little of what we now understand as sanitation, hygiene, and preventive medicine. Men lived to old age, if they did, by virtue mainly of the strength of their innate constitutions, and their good luck in avoiding fatal accidents. At the present time hospitalization, the science and art of medicine and public health, and general sanitation keep many persons alive well into middle age who would in those days have died much earlier because of a lack of constitutional ruggedness. Altogether the data of Fig. 5 seem highly significant in relation to the hypothesis suggested above as to the reason for the difference between *Drosophila* and man in respect of mortality curves.

ACCIDENTAL DEATHS

The tacit assumption in all the foregoing is that each of the 11,772 flies comprised in the four life tables died a natural death, and that the time of death (or duration of life) was in each case determined fundamentally by internal factors, since the environment was substantially a constant for all.

For certainly more than 98 per cent. of the flies this assumption is unquestionably true. But in view of the possibility that some few of the flies might be dying accidental deaths by drowning in the moisture, which sometimes collects on the surface of the food, it was thought worth while to attempt to prevent the collection of moisture in some of the bottles and compare the duration of life of flies kept in such bottles with that of flies kept under the ordinary conditions. Accordingly, bottles of food were prepared by putting discs of several layers of a very absorbent paper, Zorbik, in the bottom of the bottle and then pouring in the food and letting it solidify

on as steep a slant as possible, so that any moisture formed would drain down and be absorbed by the paper in the bottom of the bottle. Flies of generation 5 from four different lines were used in the experiment, two normal wild type lines, one of Old Falmouth stock and one of New Falmouth stock, and two lines of Quintuple stock. Flies were taken out from stock bottles of these lines as they emerged every day, beginning March 18, 1921, and continuing through April 4, putting hatches of alternate days in the specially prepared bottles and the other hatches in ordinary bottles. The flies put into the specially prepared bottles were of course kept throughout their lives in such bottles, and the controls in ordinary bottles. Table VI shows the l_x lines of the four groups of flies—long-winged with paper and slant food, long-winged without paper and food surface horizontal, short-winged with paper and slant food, short-winged without paper and food surface horizontal. Distributions have been made for the four lines separately, and for the sexes separately, but since they all show the same results the separate distributions are not given.

The data of Table VI are shown graphically in Fig. 6.

It is evident that there is no definite or marked difference between the slant food group and the other. Such differences as do appear between the l_x lines in the two cases are only of the order of magnitude which might readily appear in random sampling. This is indicated in another way by the data of Table VII.

In the case of the short-winged flies the difference in the mean is plainly not significant. In the case of the long-winged flies the difference is 2.96 times its probable error. One would expect a difference as great as this or greater to occur from chance alone only 4 to 5 times in every 100 trials, so that the difference is here getting on towards the magnitude where it must be regarded as certainly significant on purely statistical grounds. But the difference is in favor of the horizontal food without drainage, and *against* the food with drainage.

TABLE VI

SURVIVAL DISTRIBUTION OF FLIES UNDER DIFFERENT CONDITIONS AS TO SURFACE MOISTURE ON FOOD

Age	Short-winged Flies		Long-winged Flies	
	With Paper and Slant Food	Without Paper. Food Surface Horizontal	With Paper and Slant Food	Without Paper. Food Surface Horizontal
1	1,000	1,000	1,000	1,000
3	983	1,000	—	—
6	913	951	996	1,000
9	804	857	—	—
12	712	774	974	982
15	651	660	—	—
18	556	498	898	912
21	469	442	—	—
24	346	355	834	832
27	257	302	—	—
30	182	234	796	799
33	115	147	—	—
36	84	83	675	770
39	59	38	—	—
42	22	15	547	661
45	17	4	—	—
48	14	0	408	482
51	8	—	—	—
54	3	—	294	314
57	0	—	—	—
60	—	—	204	230
63	—	—	—	—
66	—	—	94	157
69	—	—	—	—
72	—	—	34	120
75	—	—	—	—
78	—	—	23	51
81	—	—	—	—
84	—	—	15	7
87	—	—	—	—
90	—	—	4	0
Absolute number of flies.	(265)	(274)	(358)	(265)

On the whole it seems perfectly clear that these experiments give no justification for going to the considerably greater trouble of preparing this food so that there is drainage from its surface. As a matter of fact the drainage of moisture is never entirely complete even with

TABLE VII

MEAN DURATIONS OF LIFE CALCULATED FROM THE DATA OF TABLE VI

	Mean Duration of Life		Difference of Means	Diff. / P.E. Diff.
	With Paper and Slant Food	Without Paper and Slant Food		
Long-winged	43.80± .73	46.91±.75	3.10±1.05	2.96
Short-winged	20.10± .40	20.58±.42	.48± .58	.83

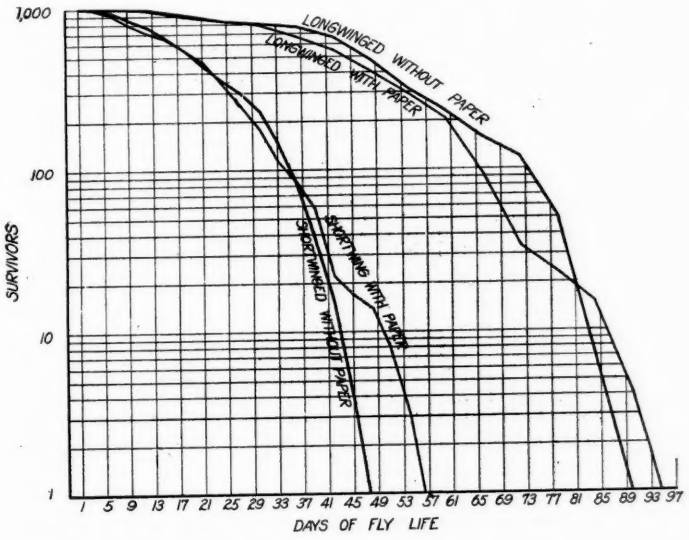

1,000
100
10
1
SURVIVORS
LONGWINGED WITHOUT PAPER
LONGWINGED WITH PAPER
SHORTWINGED WITH PAPER
SHORTWINGED WITHOUT PAPER
1 5 9 13 17 21 25 29 33 37 41 45 49 53 57 61 65 69 73 77 81 85 89 93 97
DAYS OF FLY LIFE

FIG. 6. Comparing survival (l_x) lines under different food conditions explained in text.

the slant food and absorbing paper. Small drops still cling in some cases to the agar, and a fly might drown in such a drop just as well as in a similar drop on a horizontal surface. The important point is that this experiment confirms our general experience in this work, namely, that accidental deaths occur so extremely rarely under our conditions that they do not appreciably affect the results.

SUMMARY

This paper is the first in a series of experimental studies on the factors influencing the duration of life in *Drosophila melanogaster*. An account of the experimental technique used in these duration of life studies is presented. Four complete life tables for *Drosophila* are given, and it is shown that this organism follows quantitatively the same general law in respect of the distribution of its mortality as does man. As this work deals only with the duration of imaginal life in *Drosophila* there is no component in the life tables corresponding to the mortality of infancy and childhood in man. It is shown that there are wide differences in duration of life in different stocks of *Drosophila*, and that the basis of these differences is hereditary and not environmental. The *Drosophila* survival line of the life table (l_x) runs in general throughout its course between human survival lines of (*a*) the present time, and (*b*) about the beginning of the Christian era (Macdonell's data from Roman Africa), the curves being superposed on the basis of the omission of the human mortality of infancy and childhood.

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INHERITANCE OF CANCER IN MICE

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IN the following we shall give a summary of our investigations into the part heredity plays in the origin of cancer. Our interest in this problem dates back a considerable number of years. In 1899, in conjunction with Dr. Jobson, we made our first observations on the endemic occurrence of cancer at the Stockyards in Chicago. At that time we found cattle coming from a certain ranch in Wyoming especially prone to have cancer at the inner canthus of the eye (1). A few years later we observed an endemic occurrence of sarcoma of the thyroid in a family of rats. In this case we pointed out that the circumstances under which these sarcomata originated pointed to a hereditary condition rather than to an infection. At that time (1904)[1] we referred to the desirability of investigating experimentally this endemic occurrence of cancer (2) and we had particularly in mind an analysis of the etiological factor in breeding establishments of mice or rats. Such an opportunity presented itself a few years later in the breeding establishment of Miss Lathrop in Granby, Mass. A preliminary investigation here revealed the fact that cancer occurred with much greater frequency in certain inbred strains than in others, and that no indications whatever could be found of cage infection or direct infection from animal to animal. We concluded in 1907 on the basis of these observations that there existed a hereditary predisposition which was responsible for the endemic occurrence of cancer (3). At that time we planned further much more extensive experiments on the mode of hereditary transmission of cancer in following isolated families and strains of mice through several generations and in study-

[1] A paper presented in abstract before the II Intern. Congress of Eugenics, New York.

ing the effect of hybridization on the cancer rate. This was made possible to us in 1910 through the interest which Miss Lathrop, who bred a very extensive stock of mice, took in these plans and with her cooperation we carried out these breeding experiments from 1910 on through a considerable number of years. In the following year we published the record of a cancer family of mice which we had observed through several generations (4). Altogether we carried out observations on approximately 12,000 female mice reaching the cancer age which we followed throughout the whole period of their life and which were observed through successive generations. Many of them were used for hybridization.

In the meantime E. E. Tyzzer had published in 1907 and subsequently studies of the inheritance of cancer in mice (5). This author found indications that heredity may play a part in the etiology of animal cancer. Somewhat later Murray undertook similar studies in which he used methods similar to those previously employed in the study of human cancer. This author compared the frequency of cancer among individuals whose mother or grandmother had cancer on the one hand, and among those in whom cancer had not been observed in the direct ancestry but may have occurred in the great grandparents (6). Murray found on the average about 20 per cent. of cancerous mice among those whose direct ancestors had suffered from cancer and 11 per cent. among those whose direct ancestry had been free from cancer. In certain age classes the cancer rate of both kinds of mice showed only a very slight difference and in one age class the cancer rate was even higher in those whose direct ancestry had not been affected by cancer.

These results as well as our previous studies and some occasional observations of Albrecht and Hecht and of Spronk and a few others made very probable the significance of heredity in the etiology of cancer.

Nevertheless there were opposing views such as those of Borrel and others who referred the endemic occurrence

of cancer not to heredity, but to infection, and as late as 1910 Bashford expressed the opinion that heredity has no significance in the causation of cancer.

We believe that our investigations which were carried out with the cooperation of Miss Lathrop established the importance of heredity in the etiology of cancer beyond doubt, and they point more accurately to the mode of inheritance and the interaction between heredity and other factors (7). Subsequently Miss Maud Slye began to use a large stock of mice which she had collected for biological purposes for a similar study of heredity of cancer in mice (8). While in general her conclusions as to the significance of heredity in cancer agree with ours and are thus confirmatory of ours, she has extended her researches in various other ways and has made valuable contributions. In regard to certain questions our conclusions differ. To those we shall have occasion to refer in the following pages.

The following is a summary of our main conclusions:

1. The cancer rate of each strain of family is a definite characteristic of this strain and is transmitted by heredity to successive generations. The differences in the tumor rate in various strains are very pronounced; the tumor rate may vary between zero in certain strains and almost 100 per cent. in others. All intergrades may be found. To cite a few examples of tumor rates of various strains: English 67.6 per cent., European + I daughter of No. 10 72 per cent., 344 + 328 = 79 per cent., London 28 per cent., No. 8 27.5 per cent., 8 + German 34 per cent., Cream 5.9 per cent., European 9 per cent., German + 8 0 per cent. (344 + Black Cream) + Cream 0 per cent.

While these strains represent composites, they are on the whole in so far homogeneous, as in the large majority of cases substrains showed similar tumor rates. Thus in the case of the English and Cream, for instance, numerous substrains showed the typical tumor rates. In a number of cases individual families were separated and followed, and on the whole their tumor rate agreed very

well with that of the main strain. Certain deviations must of course be expected in the case of relatively very small numbers of individuals obtained in case of an individual family or small substrains, and yet quite frequently even small substrains or families agree in their tumor rate with the main strain.

We may give some examples of the comparative tumor rates of the main strains and of the substrains: The strain $8\frac{1}{2} + 328$ had a tumor rate of 56.4 per cent. There was among them a family No. 1075 consisting of ten females reaching an age which permitted inclusion in these records. They had a tumor rate of 60 per cent.; another family of this strain (No. 1,113), consisting of sixteen such females, had a tumor rate of 88 per cent. Family 782*a* (22 females) had a tumor rate of 68 per cent. Among the substrain English Sable which had in the corresponding generation an average tumor rate of 75 per cent., there was a family No. 437 (27 females) with a tumor rate of 89 per cent. The same correspondence is found in others and among them low rate strains. In certain cases, however, certain substrains of families can be split off which differ in their tumor rate. Thus at an early period of inbreeding there were split off from the English strain two substrains with low rates: English Silver and English Silver Fawn, with tumor rates of 8 per cent. and 12 per cent., respectively.

From the strain London (tumor rate 28 per cent.) two families were branched off which showed very different rates: London Blue and White (31 females) 55 per cent., and Family 481 (25 females) 0 per cent. But these are not the usual occurrences. A correspondence between main strains, substrains and families is the usual finding.

We see then that all kinds of intergrades in the tumor rates occur in different strains, substrains and families and that these are on the whole constant and characteristic of strains and families.

2. These differences in rate persisted through successive generations in the majority of our strains with a sur-

prising regularity. Thus for instance, in the strain London, the earlier generations (120 female mice) showed a tumor incidence of 27 per cent., in the intermediate generation (61 females) the figure was 38 per cent. and in the later generation (197 female mice) 28 per cent. Similar conditions were found in a number of our strains. In certain strains, however, variations in the tumor rate did occur. While some variations may of course be expected, in case the number of mice considered is very small, there occurred in addition changes which can not be attributed to this factor.

In the majority of cases in which these latter changes did occur in our stock, they consisted in a decrease in the tumor rate in later generations; in a few cases only there occurred an increase in the tumor rate. These changes were in all probability due to two factors: (*a*) In certain families and strains as a result of long continued inbreeding a gradual decrease in fertility and vigor occurred. Associated with this change was in certain cases a noticeable decrease in the tumor rate. Especially in the strain No. 8 there seemed to be a connection between loss in resistance to disease and fertility and the decrease in the tumor rate. This strain was inbred for seventeen generations and the changes in the tumor rate seemed to occur step by step in correspondence with the progress in inbreeding. Under those conditions the connection between inbreeding and change in the tumor rate appears the most probable explanation, although it can not be considered as definitely proven as yet.

(*b*) Various factors caused a selection to take place within the strain; certain families died out, while others, which happened to be more resistant to a certain disease, survived, propagated and thus gained a preponderance. These surviving families differed sometimes in appearance, or in vigor, in the behavior towards certain inoculable tumors. Such changes were accompanied in certain cases by a change in the tumor rate. In the majority of our cases a decrease occurred; in a few cases an increase;

but even in such cases the increase was moderate; there was never observed among our material a sudden transition from a low to a high rate tumor strain. The increase as well as the decrease in the tumor rate was caused by the same factor; whether one or the other should prevail depends more or less on chance, and in different material the number of strains showing the one or the other variation may be expected to differ. It has been maintained that in strains which have been inbred for a long period of time and in which a decrease in fertility occurred as the result of the inbreeding, development of cancers takes the place of the lost fertility. In inbreeding cancer replaces reproduction, as it has been expressed by Maud Slye. In our material such a substitution did not take place; in inbreeding mice vanishing fertility was not replaced by the development of cancer under ordinary conditions. Inbreeding does not lead to an increased cancer rate.

3. If we cross strains with a similar tumor rate, the offspring inherits the tumor rate common to both parents; if both parents differ in tumor rate, the tumor rate of the offspring is on the whole intermediate between those of the parents. But all degrees of intermediacy are observed. In our material the number of strains in which the rate of the parent with the higher tumor incidence dominated was on the whole greater than the contrary one.

We selected for our hybridizations especially strains which differed markedly in their tumor rate and other characteristics and which had been followed over long periods of time and had been found consistent in their behavior. The English as a representative of a high tumor rate strain and the Cream as a representative of a low tumor rate strain were especially suitable for this purpose. In the majority of cases we selected few individuals for hybridization, either one male and one female or one male and several females. We followed the offspring through several generations. The near relatives of the

individuals used for hybridization were observed as to their tumor incidence and generally found to behave in a way characteristic of their strain.

Sometimes we hybridized sisters with the same male, or we used consecutively the same male with females from strains which differed much in their tumor rate. The results in the hybrids could usually be foreseen from the known tumor rate and tumor age of the parent up to a certain point of variability. The cases in which the strains used for hybridization had a similar tumor rate could be considered as controls. Here a similar tumor incidence ought to have appeared in the offspring and this is what usually occurred.

As we stated above, the results of hybridizations are typically intermediate, but the rates and tumor ages of the crosses may in some cases approach the parent with the higher rate, in other cases the parent with the lower rate.

We shall cite two examples, where the offspring resembled the parent with the higher tumor rate. (1) A son of a tumor mouse No. 240, belonging to the strain 8 + German, was mated to a White Cream female. 8 + German was a strain fairly rich in tumors and the particular family used had a tumor rate of 43 per cent. The tumors appeared early in life. In the White Cream used in this case tumors were extremely rare and they appeared late in life. Among the offspring 9 female mice lived long enough to be included in the records. Of these 9 mice, 5 died with tumors, 1 in the first and 4 in the second age period. In this case the influence of the father is undoubtedly very marked. In the Cream strain such a tumor rate was never observed even among isolated families. The tumor age of the hybrid is, however, in this case probably affected by the mother.

(2) In the second case which we wish to mention, an English Sable male belonging to the fourth generation of English Sable, who have normally a very high tumor rate, was mated to 3 females belonging to the substrain

Cream Y. In the substrain Cream Y the tumor rate had been zero. Four generations of the offspring were observed comprising altogether 68 female mice which reached an age sufficient for inclusion in our records. Thirty-six of these mice, that is, 53 per cent., died with tumors, 11 of these in the first age period. This is a record which comes near that of the English Sable. These as well as numerous other experiments seem to us more in harmony with the conclusion that multiple factors underlie the hereditary predisposition to mammary cancer in mice than the view of Maud Slye who maintains that the factor for mammary cancer in mice is a recessive monohybrid.

(4) The age at which tumors appear is just as characteristic of individual strains as the tumor rate. The tumor age is also transmitted by heredity. In some strains tumors appear relatively early, the percentage of tumors appearing in the first age period of life comprising the first twelve months is considerably greater than in others; and this characteristic is on the whole just as constant in the strains as the tumor rate as a whole.

We can distinguish two factors in the inheritance of the tumor age: (*a*) In general in the strains with the higher tumor rates the tumors appear at an earlier period of life than in the lower tumor rate strains. This comes out very clearly when we divide all the strains into three classes, those with a tumor incidence above 40 per cent., the high tumor rate strains; those with a tumor incidence between 20 per cent. and 40 per cent., the medium tumor rate strains, and those with a tumor incidence below 20 per cent., the low tumor rate strains.

If we determine in each class the percentage of tumors appearing in the different age periods, we find that the tumors appear the earlier in life the higher the tumor incidence and the difference between the different classes is quite marked.

There is in our case a definite relation between the factors, tumor age and tumor rate. We can interpret this

relation by assuming that a certain average quantity is inherited in the individuals of different strains which determines the intensity in the tendency towards the development of tumors. This intensity may depend on the average number and character of multiple factors favoring tumor growth which is characteristic of a strain. A special kind of factors or a larger number of factors causes both a higher incidence and an earlier appearance of cancer in a certain strain.

(*b*) In addition to this intensity which is a characteristic of the strains in general, there is a peculiar tumor age in certain strains which is independent of the tumor rate. Strains with a similar tumor rate may differ in their tumor age, and in hybrids tumor rate and tumor age may be inherited separately in the offspring. Thus two of our high tumor rate strains formed by the crossing of the same male (European 151) with two sisters (first and second daughter of No. 10, respectively) with tumor rates of 72 per cent. and 54.5 per cent., respectively, have relatively late tumors, in both only 15 per cent. of the tumors appearing in the first age period. In the Cream-English hybrids the tumor rates in two strains were similar and approximately intermediate, but in one of them the tumor age approached the late one of the cream parent; in the other it was nearer the early one of the Eglish parent.

We may therefore assume that in addition to the sum total of multiple factors which determine at the same time age and tumor rate, there are special factors which determine tumor age.

5. In general the cancer rate in mice is not a sex-linked character. Either the cancer rate and age of the father or mother strain may predominate in the cancer rate of the offspring. This fact does not, however, exclude the possibility that in certain cases a sex-linked factor may enter as one of the multiple factors which in all probability determine the inheritance of cancer. Certain of our observations suggest such a possibility. We found, for

instance, that in the Cream-English hybrids the mother strain was considerably more often predominant than the father strain; in addition we found that in reversing a cross different results were obtained in accordance with the difference in the tumor rate of the mother strain. It is, however, possible that these occurrences are chance phenomena and we offered this interpretation merely as a suggestion.

6. Our investigations make it possible to express in a quantitatively definite manner the hereditary tendency to cancer in individual strains of mice, the figures varying in different strains between zero and 100. This hereditary tendency is, however, not a simple quantity, but composite, because

(*a*) The hereditary disposition to cancer is probably due to the cooperation of multiple factors. The results of hybridization, which essentially were of an intermediate character, the fact that all kinds of intergrades between father and mother strain exist and that all possible variations in the hereditary tendency to cancer exist in different strains, and that the hereditary tendency determining the cancer age is not entirely identical with the tendency expressed in the cancer rate, very strongly suggest this conclusion. Variations in the number and character of the multiple factors in the different individuals may be responsible for the variations in intensity which determine the tendency to cancer in individuals, and different strains may differ as to the mean in the distribution of the factors among the individuals belonging to the strain. Thus we may assume that the strains English, $8\frac{1}{2} + 328$, European + I daughter of No. 10 have on the average a greater number of factors than the individuals of the strain Cream and German + 8 and many others; and it would be conceivable that in many cases a tumor mouse belonging to the strain English differs from a tumor mouse in the strain Cream, the former often exceeding the minimum of factors necessary for the production of tumors.

As far as the ordinary mammary cancer of the mouse is concerned, no definite proof has so far been brought forward to support the view that the hereditary tendency to cancer is due to the presence of a simple recessive factor.

(*b*) There is hidden in the figure expressing the tendency towards the development of cancer a second factor which is variable; namely, the activity of the ovary. In all the strains the realization of the hereditary tendency to cancer presupposes the activity of the internal secretion of the ovary. Without this cooperation no cancer can originate. With the full activity of this factor the hereditarily transmitted character for intensity of cancerous tendency determines the upper limit of the cancer rate. Again the intensity of this ovarian factor can be expressed in a quantitative manner, the quantity in this case representing the time during which the ovarian internal secretion had a chance to act. If through castration in the early stages of adult, sexually mature life, at the age of three to four months, this internal ovarian secretion is eliminated in mice, mammary cancer is practically prevented from appearing even in normally high tumor rate strains. The longer the ovarian function has a chance to act, the more the cancer rate increases up to the range which is given in the figure for the hereditary tendency to cancer. While we can thus experimentally lower the cancer rate of any strain, we do not so far know of a method which would permit us to raise the cancer rate above this point. The latter is almost reached if castration occurs at the age of eight to ten months. Suspension of breeding also diminishes somewhat the cancer rate in the great majority of the cases, but to a very much less extent than the exclusion of the internal secretion of the ovary, which latter is the true realizing factor, the cooperation of which is necessary. In one strain in which through segregation of the female mice breeding had been prevented the cancer rate was even higher in the non-breeding than in the breeding mice (9). Injury to the mamilla by the suckling young which Maud Slye believed

to be the external stimulus leading to the development of cancer in mice can therefore not be an important factor in the causation of mammary cancer in this species of animals. On the other hand, our demonstration of a certain influence of breeding on the cancer rate in mice adds another, though minor, factor to the internal secretion of the ovary, which latter represents, as we stated above, the principal realizing factor. Secondary realizing factors may therefore be added to this primary factor.

In principle, conditions are probably similar to what we determined in the case of the typical mammary cancer of the mouse in all other kinds of cancer. But we have to assume that the internal secretion of the ovary is substituted in other cases by other variable factors, which may be either internal secretions of a different kind or external stimulations. The latter play, as is well known, a very important rôle in the origin of cancer. They represent in addition a quantity which can be increased at will in contradistinction to the internal secretions and other inner factors. Thus through the use of external stimulation it may be possible to increase at will the cancer rate in certain kinds of cancers; in this way the hereditarily fixed intensity may become entirely obscured. Yet it can not be doubted that after all this factor is present even in these latter kinds of cancer, the best representative of which is perhaps the Roentgen ray cancer in man.

7. Thus it has become possible to express in a quantitative way the tendency to a disease, cancer. This tendency is due to the interaction of two main factors, both internal, the one hereditarily fixed and the other accessible to experimental variation. Both factors combined are more than the predisposition to cancer; they are in the case of this particular kind of cancer its essential cause. There may be, as we have seen, other factors superimposed upon these primary factors, like the effect of pregnancy; but they are not necessary, and the first two factors suffice for the development of mammary cancer in

the variable numbers which are characteristic of the different strains of mice.

8. Is it possible to associate the hereditary tendency to cancer with the other factors characteristic of particular individual mice or of strains of mice? We found in certain cases that from main strains substrains could be detached which differed from the main strain not only in color, but also in the tumor rate; the most noteworthy cases of this kind are the English Silver and Silver Fawn substrains, detached from the main English strain at an early period of inbreeding. In this case the tumor rates differed in a very pronounced manner from that of the main strain. But the connection between color and cancer rate or age is in this case, as in some other cases, an accidental linkage. There is no real causal connection between the color and the factors that determine cancer. It is apparently similar in the case of other characters such as vigor, prolificity, size and rapidity of growth. We find strains of all kinds among the high as well as the medium and low rate tumor mice. This comes out quite clearly in the case of the various English-Cream hybrids. Here the tumor rate and age may be quite similar, namely, intermediate in different crosses, and yet some of these strains may be vigorous, others frail; some prolific, others poor breeders. In crosses certain characteristics, such as wildness or tameness, vigor and resistance to disease, or frailty, prolificity or the opposite, are inherited, just as the cancer rate and the cancer age; but these characters may be distributed among the hybrids independently of the predisposition to cancer. However, it so happens that some of the most prominent low rate tumor strains are poor breeders, slowly growing, although vigorous mice, while some of the high rate tumor strains are prolific, more rapidly growing; but this relation does not seem to hold good in all cases and may therefore not be causal. Quite recently T. B. Robertson observed among his mice that especially the rapidly growing individuals were prone to become cancer-

ous and he believes that a causal connection between the developmental rate and tendency to cancer exists.

9. There may, however, possibly be an exception to this independence of the hereditary transmission of the tendency to cancer. As we have stated, we arranged our various strains of mice in three groups, in strains with a high, with a medium and low tumor incidence, and we found that in these three groups the cancer age varies *pari passu* with the decrease in cancer rate. If we now determine in these same three groups on the same percentage basis the age of death from all other causes taken together except cancer, we find the differences between the three groups considerably less than if we compare the percentages of the cancer age. There is, however, a distinct difference. In the group of the high cancer rate strains the age of death from all other causes but cancer is decidedly earlier than in the medium and low rate cancer strains. The difference between the medium and low cancer rate strains is very slight, very much less than that between the high and medium rate tumor strains, but this slight difference is again in the same direction. This relationship between cancer rate and age of death from other diseases may be explained in two ways: (*a*) We may assume that the mice dying from cancer are the strongest, most resistant individuals of the family or strain and those which are left back are therefore relatively less resistant to disease; the higher the cancer rate in a strain, the less resistant are the mice not dying from cancer, and the earlier, therefore, their age of death from other causes. Or (*b*) the majority of the strains in which the cancer rate was high happened to be less resistant strains and therefore the average age of death from other diseases is earlier. The average difference between the medium and low rate tumor strains, as far as general power of resistance is concerned, happened to be very small. Although it is perhaps impossible to decide definitely between these alternatives, we believe the second one to be much more

probable. If the first alternative were correct, we should expect to find a decided difference also between the age of death from other causes than cancer in the medium and low cancer rate strains. Here the difference is almost negligible. Furthermore, there are some strains with a very high tumor rate, but in which the rate of death from cancer in the first age period is relatively small. In those strains the resistant individuals would therefore be spared by cancer in the first age period; thus the resistant individuals would not be eliminated and the age of death from other causes should accordingly be late in these strains. Actually we find in such high tumor rate strains an early age of death from other causes. We may therefore conclude that in the material on which we base our conclusions the large majority of the high rate tumor strains were strains with a low general resistance to disease. While, as we have stated above, a high or low degree of resistance may be associated with either a high, a medium or a low cancer rate, this association of a low degree of resistance with a high cancer rate in a prepondering number of strains may possibly be more than a coincidence. Maud Slye states that cancer strains are the strongest strains, a conclusion at variance with our experience.

10. The tendency to die from other causes than cancer at a certain period of life, the resistance to disease in general, is also hereditarily transmitted, but as we have stated above it varies among different groups of strains much less than the predisposition to cancer. This should be expected if we assume that there exists besides a general power of resistance a special resistance or predisposition to individual diseases, and that the latter may vary among different strains and may thus to a certain extent balance each other in various strains.

Again the tendency to die from other diseases than cancer at a certain period of life depends upon the cooperation of the generative organs; but while in the disposition to mammary cancer the internal secretion of

the ovary is the main factor and suspension of breeding plays only a subordinate rôle, in the case of resistance to other death producing conditions, the suspension of breeding seems to be the main factor and the elimination of the ovarian function only a subsidiary factor which merely acts through the suspension of breeding which it calls forth or in which at least the suspension of breeding is by far the more significant factor. We found that the differences in the tendency to die from other causes than cancer which we observe normally between different strains of mice are entirely or at least to a great extent eliminated in mice which are prevented from breeding. All those strains in which breeding is prevented become long lived. If a difference in the duration of life should still exist between different non-breeding strains, it must be very much smaller than that between breeding strains. Furthermore, the difference in the longevity between non-breeding mice and castrated mice is likewise very small and this is the reason why we conclude that castration prolongs the life of mice mainly through its effect on breeding. As far as the cancer rate is concerned, on the other hand, we have shown that castration at an early age is much more effective than prevention of breeding.

11. In man it has been observed by several authors that in older individuals suffering from cancer a multiplicity of slight malformations, often due apparently to a misplacement of embryonal tissue, or a multiplicity of benign, or rarely even of malignant, tumors could be observed. Similar observations were made more recently by Goodpasture in the case of old dogs. It is usual to attribute these findings either to an inherited tendency to tumor formation in general in which imperfections in embryonal development play a certain part, and in which, as a result of this general tendency, various kinds of tumors develop in the same individual in its old age, or by some authors emphasis is laid on the importance of old age as such in the etiology of cancer; old age is sup-

posed to bring about a multiplicity of tumors or corresponding malformations.

In a similar manner Maud Slye states there is in mice inherited a general tendency to cancer. In hybrid strains, according to this author, this general tendency finds expression in the first hybrid generations in a tendency to develop sarcoma, while in subsequent generations more specialized tissues are affected which develop into carcinoma, and in still later generations multiple tumors are prone to appear.

We have not been able to observe such a cycle in our strains of mice. We had uniformly in all generations to deal with mammary carcinoma and in many autopsies which we made of tumor mice we failed to observe other kinds of tumors. This does not exclude the possibility or even probability that occasionally lesions may have been present in other mice which were tumors of a different kind. We described, for instance, a beginning squamous cell carcinoma in a mouse afflicted with a mammary cancer about 10 years ago; but on the whole such occurrences were rare and they could not be interpreted as due to the inheritance of a general tendency to cancer; in each case external factors would then at least partially determine which particular expression this general tendency should find.

In our strains there was inherited essentially, not a general tendency to tumor formation, but a specialized tendency to cancer of the breast. This does not exclude the possibility that in certain strains a tendency to the development of another kind of tumors may have been inherited side by side with the tendency to mammary cancer. In favor of this conclusion we may cite the experiences in cases of the so-called endemic occurrence of cancer, as, for instance, the cancer of the inner canthus of the eye in cattle observed by us in 1899, the cancer of the scrotum in the rat observed by Hanau, our observations of sarcoma of the thyroid gland in the rat. All these are instances of the inheritance of specific kinds of cancers.

The most striking confirmation of this view has in recent years been funished by Miss Slye, who discovered certain families of mice in which a tendency to special cancers, as, for instance, cancer of the liver, was inherited. We therefore conclude that inheritance to cancer consists in general in a tendency to the inheritance of a particular kind of cancer. This agrees also with the results of Miss Stark, who found in *Drosophila* two specific kinds of inheritable, tumor like formations originating by mutation.

12. Our continued investigations have thus borne out our earlier conclusion that the endemic occurrence of cancer among animals is due to this hereditary transmission of the disposition to cancer. In addition, infection with certain metazoon parasites which act as an external stimulus comparable to the action, for instance, of Roentgen rays, may play a part in certain cases; but even here the metazoon parasites seem to act on a basis of hereditarily transmitted disposition. The observations of Fibiger, with which the recent findings of Rohdenburg are in agreement, suggest this conclusion.

13. While these statements apply directly only to animals, the evidence on hand makes it probable that, in principle, conditions are similar in man; here also in all probability one or more factors are hereditarily transmitted which determine the intensity in the tendency towards cancer development. In man this tendency has, however, in many cases been more or less equalized among different families as a result of long continued cross breeding (10). Wherever this factor can be eliminated as among different races which remained relatively pure or among a very stationary population, as in certain parts of Norway, the evidence points to the conclusion that here too marked differences in the tendency towards cancer exist in various strains and races (11). Even among the ordinary population some occasional striking findings very strongly suggest this view.

Furthermore, Davenport (12) has shown that the tendency to neurofibromatosis is hereditarily transmitted as

a dominant. Similarly, the tendency to certain other tumors is undoubtedly inherited. The recent statistical studies of C. C. Little make it very probable that an inherited predisposition to cancer plays a part in human cancer in general (13).

As to the increase in the cancer rate which seems to be so general an occurrence, we may suggest that, so far as it is not due merely to improved diagnosis, it could be referred to a greater frequency in the dominance of the parent with a tendency to a higher tumor rate in the offspring.

As we stated above, such a condition of dominance was observed among our hybrid strains.

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DEVELOPMENT OF GONADS AND TRANSFORMATION OF SEX IN THE FROG

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IN the following paper I intend to give a summary of the sexual problems in the frog, as they result from descriptive and analytic research. Richard Hertwig's successful experiments on sex-determination have directed the attention of investigators to this object. From 1911 to 1913 the writer was fortunate enough to have the opportunity of working in Hertwig's laboratory and since then the investigations have been continued at the zoological laboratory of Basle University. The present paper is a brief summary of the development of the sexual characters. In a later publication we hope to explain the experiments on sex-determination and to describe the chromosome cycle.

In the number of this journal for July–August, 1920, Dr. W. W. Swingle has published an interesting study on "Neoteny and the Sexual Problem," and in the February number of the *Journal of Experimental Zoology* the same author has described in greater detail the developmental history of the male gonad of *Rana catesbiana*. In both communications Swingle has disputed the correctness of the work of the Hertwig school. The following explanations may be regarded as a reply to Swingle's critical remarks.

A. THE SEXUAL GLANDS

1. *Morphology of the Undifferentiated Gland.*—The gonad of larvæ 22 mm. in length, just before sex-differentiation takes place, has the following structure. A simple germinal epithelium encloses the central primitive gonad cavity. Five to seven sex cords, budding from the

mesonephric blastema at regular distances, fill the cavity as shown in Figs. 1 and 2.

FIGS. 1. AND 2. Transverse sections through the two types of undifferentiated glands. Larvæ 22 mm. total length; 12 days old.

2. *The Ovary.*—The ovary is formed by direct development from the indifferent gonad. In consequence of a rapid increase of the germ cells by mitotic divisions the germinal epithelium thickens. In larvæ of 30–35 mm. total length the germ cells begin to be arranged in ovocysts. After a few simultaneous mitotic divisions the cells of the cysts enter the maturation stages. As Fig. 3 shows, the first cysts are found in the distal part of the gland. Towards the end of the larval period the formation of the oldest ovocysts is disintegrated. Each ovocytis having passed through the stages of pseudoreduction (synizesis, leptotene, pachytene) is surrounded by follicle cells and now enters the second period of growth. The nucleus increases considerably in volume and in the growing protoplasm numerous vitellogen granules appear (Fig. 7, sec. Oc.). Yolk is however not formed normally before the third or fourth season. The sex cords are of greater importance only in the male gonad, forming the testicular interstitium and the rete apparatus—while in the ovary each cord develops into an ovarial sac. As Fig. 1 shows, a little slit may appear already in the undifferentiated gland. More such are found when ovocysts

begin to be formed in the germinal epithelium (Fig. 3, *c*). But during the stage of metamorphosis the slits unite and form only one cavity: the secondary gonad cavity or ovarial sac. In consequence there are in each ovary 5 to 7 sacs, forming the thin gonad endothelium (Fig. 7, I, II).

FIG. 3. Section through a young ovary. *C* = secondary gonad cavity. *O* = ovocysts. *F* = follicle cell. Tadpole 38 mm. total length.

FIG. 4. Transverse section through the earliest developmental stage of the testicle. Migration of the germ cells. *sp* = first germ cell in the sex cord (spermatogonia). Tadpole 22 mm. total length; 12 days old.

Characteristics: 1. *Persisting peripheral germinal epithelium.* 2. *First stages of ovocytes in the larval period.* 3. *Occurrence of a second growth period.* 4. *Presence of ovarial sacs.*

Therefore, precocious ripening of germ cells is not my "chief criterion of sex-differentiation," as Swingle says. On the contrary the other three are more characteristic, because they are completely absent in the male line!

3. *The Testis.*—In larvæ 22 mm. in length great differences in the size of the sex cords are found, as seen from a comparison of Figs. 1 and 2. Animals with the stout ones are undergoing transformation into males.

The testis is not formed by direct development from the indifferent gonad. *Its development begins with a change in the position of the germ layers.* The peripheral germinal epithelium having disintegrated, the germ cells cross the primitive gonad cavity and enter the sex cords (Figs. 4 and 5). The follicle cells migrate with the gonia

and only the simple peritoneal epithelium remains (Fig. 5).

Shortly after this migration the canaliculi seminalis are formed, at first as irregular slits, but already during metamorphosis they develop into characteristic radiating tubes. At all times they remain connected with the sex cords. These on their part give rise to the rete vasculosum Halleri and the connections with the mesonephros (Vasa efferentia testis).[1]

In the following years the testes grow very slowly until the fourth season, when a rapid increase due to active mitotic divisions of the spermatogonia begins. The spermatic tubes become convoluted; spermatocysts are formed and in the month of July the maturation cycle begins. In August and September the spermatozoa are developed.

In only one case have I found an abortive præspermatogenesis in a three-year-old grass frog (*R. temporaria*). But as Champy has observed, similar degenerating maturation cycles are frequently formed in water frogs (*R. esculenta*) in the second year. According to Swingle precocious ripening of male germ cells in the bull frog (*R. catesbeiana*) already occurs in first year larvæ, and ripe spermatozoa are formed in second year animals.

Characteristics: 1. *Central germinal layer.* 2. *First ripening stages giving rise to functional spermatozoa in the fourth season.* 3. *The maturation divisions directly succeed the pseudoreductional stages.* (There is no second growth period.) 4. *Rete apparatus and Vasa efferentia testis.*

4. *Hermaphrodism.*—It is a strange and interesting fact that the typical development of the testis as just described is rarely observed. Under natural conditions most individuals develop first ovaries which later on are transformed into testes. During this transformation the gonads, showing the characteristics of both sexes, are hermaphroditic; but without exception the female

[1] Cf. Witschi, 1914.

characters become reduced and mostly disappear completely. In Fig. 6 we have a transverse section through a larval hermaphroditic gland. As in the young ovary (Fig. 3) the distal part contains ovocysts with ovocytes in the synizesis stage, and in the sex cord is found the second gonad cavity or ovarial sac. But the transforma-

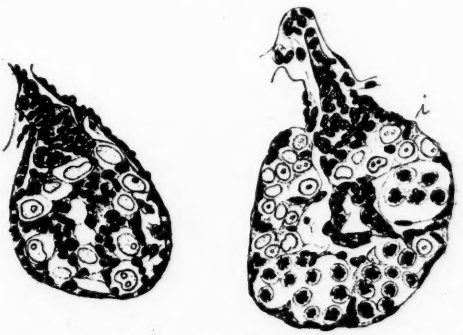

FIG. 5. Cross-section through a young male gonad. Leaved germinal epithelium. All germ cells in the sex cord.

FIG. 6. Transverse section through a larval hermaphroditic gland. Female germinal epithelium; ovarial sac. At (*i*) migrating germ cells.

tion has already begun. The sex cord is more compact than usual and its middle part is penetrated by immigrating germ cells from the basal end of the germinal epithelium. These germ cells after their entrance into the sex cords are to be called spermatogonia.

After the metamorphosis the whole female germinal epithelium undergoes degeneration (only the peritoneum is preserved) while the central testis anlage develops into a normal male gonad.

Sometimes great irregularities are observed. It occurs occasionally that one gonad undergoes the transformation of sex earlier than the other in consequence of which such animals pass through a stage of lateral hermaphrodism. Such cases have often been described and are, I believe, of the greatest interest with regard to the development of somatic sex characters. But even within the same gonad differences can be found. Sometimes the

transformation begins at one pole and proceeds continuously to the other. Fig. 7 shows a longitudinal section of such a hermaphroditic gonad. The small testicular part at the posterior end could easily be distinguished macroscopically. The fifth sex cord is almost a normal male one. Numerous spermatogonia are scattered throughout. The slits, which are seen, represent the beginnings of the spermatic tubes. At (*a*) a bridge of tissue

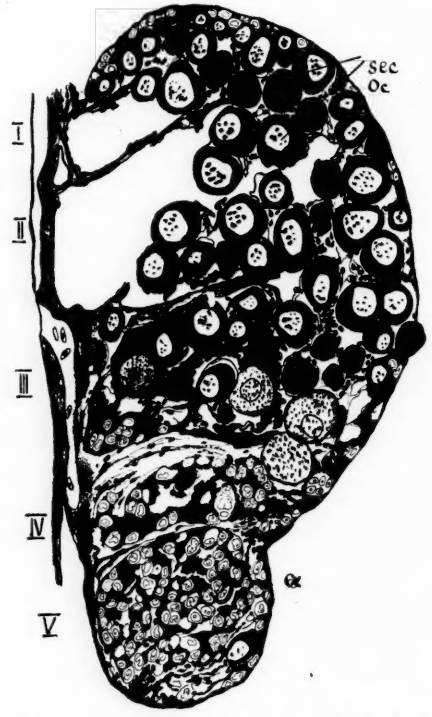

FIG. 7. Longitudinal section through a hermaphroditic gonad. Transformation of sex proceeding from the posterior to the anterior pole. *I–V* = the five sex cords. *sec. oc* = ovocytes in the second growth period.

is seen, connecting the cord with what is left of the female germinal epithelium. In the fourth cord the transformation has not progressed as far as in the fifth. The center

is occupied by the shrinking ovarial sac with a degenerating egg. The spermatogonia are not numerous but they are already dispersed throughout the wall of the ovarial sac. The germinal epithelium contains a degenerating egg and an ovocyst. The third cord shows the beginning of the process of transformation. It is greatly enlarged, but only a few spermatogonia have migrated into its basal part. Numerous degenerating eggs are scattered over the distal tissue of the sex cord. The germinal epithelium is disintegrating. The second and the first sex cords show the typical proportions of an ovary: wide and thin-walled ovarial sacs and well-developed germinal epithelium. The ovogonia adhere to the peritoneum, the larger eggs projecting inwards. Thus the walls of the sacs are folded on the outside. This preparation is taken from a first year frog, several months after metomorphosis. The transformation of sex in the grass frog often occurs in the second year but likewise is sometimes found in adult animals. Eggs scattered through the testicular tissue have been frequently observed, but ignorance of the development of the gonad has produced the belief that they were derived from spermatogonies. Recently Levy and Swingle claim that these "so-called" eggs only are enlarged ovocyte-like male germ cells; and Swingle believes that "the so-called sexually indifferent or sexually intermediate forms of the Pflüger-Hertwig school are very probably male animals whose germ cells show precocious ripening as far as the pachytene stage" (1920); and "as a consequence of this curtailment of the maturation cycle to the early stages of the process, without exception these writers (Witschi and others), being unable to differentiate male from female, concluded that all frog tadpoles first develop as females, then later half of the female tadpoles must transform into males. . . ." (1921).

It is evident that Swingle has misunderstood our previous communications. In *Rana temporaria* the ovocytes do not always degenerate after the pachytene stages but

enter the second growth period (Fig. 7). It is difficult to understand when Swingle says that R. Hertwig, Kuschakewitsch and Witschi "concluded that all frog tadpoles first develop as females." On the contrary I have (1914) described the typical (or direct) development of the testicle as it is to be observed in the alpine *Rana temporaria*. Under optimal conditions of temperature in this variety of the grass frog already after the twelfth day (larvæ 20 to 22 mm. total length) 50 per cent. males are found; in such cultures transformation of sex never occurs.

In his material Swingle has not seen this typical development of the testicle. The described first and second year males are in reality hermaphrodites. His photograph 33, plate 4 (1921) does not show a transverse section through a male but through the female gonad of a first year tadpole, characterized by the ovarial sac (secondary genital cavity) and the peripheral germinal epithelium. Photograph 34 likewise is not taken from a male gonad but from a hermaphroditic one. It shows the same stage of transformation of an ovary into a testicle as Fig. 45 in our publication (1914): representing the gross structure of an ovary, but in the thickened wall of the ovarial sac are several immigrated germ cells (spermatogonia). Photograph 35 illustrates another type of transformation, characterized by an excessive proliferation of the sex cords, as is likewise described in our publication (1914, Fig. 41).

The cytological facts described in great detail by Swingle will be discussed in another communication. They do not alter our view of the significance of the developmental changes.

If there should still remain any doubt in regard to the correctness of my interpretation the following account may help to dispel it.

B. The Somatic Sex Characters

1. *The Müllerian Duct.*—The oviduct first appears as a dense cell cord close to the lateral margin of the kidney. Already in the first sumner the oviducts from the ostium abdominale tubæ to the opening in the cloaca are completely formed. They are thin walled, contain a small lumen, and run straight backwards. In the second year they grow slowly and move away from the ureters. Ordinarily in the third year a large longitudinal growth begins and the duct now becomes folded, as is well known from the anatomy of adult frogs.

In males which show a typical development of the testicles, no Müllerian ducts of any significance are formed. On the other hand, such animals as first develop ovaries and later undergo the transformation of sex, also show regular oviducts; and these continue to grow just up to the time when the transformation of sex begins. *This parallelism in the behavior of the Müllerian ducts and the gonads furnishes definite proof that the "eggs" and "ovocytes," described by the writer, are in fact really eggs and ovocytes and that the transformation of sex is a well-established fact.*

After the transformation of sex, when the ovocytes have disappeared, the Müllerian ducts begin to shrink, but they do not disappear completely, and such reduced oviducts of various sizes are often found in adult male frogs.

Regarding the question of the relations between somatic characters and the gonads, the lateral hermaphrodites furnish most interesting information. The Müllerian ducts are always developed in correlation with the gonad of the same side. Lateral hermaphrodites show always well-developed oviducts on the ovarial side and smaller ones on the testicular side. The correlation is therefore independent of the action of hormones.

2. *The Vesicula Seminalis and the Thumb Cushion.*—The male somatic characters appear chiefly in the second year and always develop symmetrically, in lateral her-

maphrodites, both sides depending from the first formed testicle. They seem, however, not to be influenced by internal secretions, as all experiments gave negative results. We will not enter here into this much discussed problem, but refer the reader to our publication on hermaphrodism.

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THE RATE OF GROWTH FOLLOWING AN INITIAL PERIOD OF SUPPRESSION[1]

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THE present paper attempts to discuss quantitative aspects of the growth of animals which, though eventually reaching the same approximate size, reached that size at widely different ages. The data upon which the discussion rests have been drawn from the published articles of several workers in the field of animal physiology and deal with the growth, under varying conditions, of the albino rat.

When the growth of an animal is suppressed for a long time the capacity to grow persists, even beyond the period at which growth ordinarily ceases in that species. The studies of Osborne and Mendel have amply demonstrated the existence of this capacity to grow and to reach the weight characteristic of mature individuals of their species. The growth impulse is something inherent in the organism. The environment, while modifying the amount of growth, has less influence upon the specific character of the growth of organisms than has the essential constitution of the living substance. A quantitative study of the growth rate of organisms ought, therefore, to lead to considerations of a fundamental nature.

The nature of the growth rate in general is revealed by the use of a few simple equations of the first order. They show that growth proceeds at a rate similar to that of a monomolecular reaction. Robertson[2] and others have discussed growth in its relation to autocatalysis. In a recent paper I have compared the equations of slowly and rap-

[1] Paper No. 84 from the University of California Graduate School of Tropical Agriculture and Citrus Experiment Station.

[2] Robertson, T. B., *Arch. Entwicklungsmech. d. Org.*, 37: 497–508. 1913.

idly growing apricot shoots. In each case the rate was proportional to a function of the final length of the shoot. The shoots which had a greater final length at the end of the season grew more rapidly from the start than their shorter neighbors, though the growing periods of the two samples were the same. The particular point of interest lay in the fact that the equations representing the growth rates had the same value for the constant of the reaction, differing only in the value of the constant expressing the final length of the shoots.

The present paper attempts to supplement this work by investigating the growth of organisms which reached approximately mature size after being subjected to conditions which suppressed growth in early life.

In the former case the difference between the two lots was in their final size; in the present case the difference between the two lots was in the time required to make equivalent body weight.

A. The Rate of Growth of Rats on Adequate Diets

The growth of the white rat has been so completely studied by many investigators that no extended discussion of the subject is required.

The rate of growth of rats varies slightly in different lots, but in general it follows the course of a differential equation. In later paragraphs I shall show that the equations used are those which express an autocatalytic reaction. The rate at which each sex grows is quite characteristic. The females grow relatively faster in early life than the males, come sooner to maturity, and weigh less at maturity than the males.

The growth of a white rat in the first year comprises two cycles. The first cycle, covering approximately 150 days, consists of a rapid increase in the weight and size of the body. The second cycle, covering the remaining 200 days, consists of a thickening of the body and a deposition of fat. The growth of rats in each of these cycles may be expressed by the equation

$$\log \frac{x}{a-x} = K(t-t_1),$$

in which x represents the weight of the animals at time t, a represents their weight at the end of the cycle, t_1 is the time at which the weight, x, is one half a, and K is a constant.

Although the quantitative relationships of this growth rate have been ably discussed by Hatai,[3] it will be shown subsequently that there are numerous reasons for using the above-mentioned formulas for computing growth. The computation of these and other growth rates studied in this paper have been made with the aid of tables published by Robertson.[4]

Table I contains data on the growth of white rats in the first year of life. The data for the rats were taken from Donaldson's tables 63 and 64.

The equations for the growth of the animals are as follows:

Males, first cycle, $\log \frac{x}{228-x} = .0187\ (t-73)$

Males, second cycle, $\log \frac{x-220}{280-x} = .0123\ (t-213)$

Females, first cycle, $\log \frac{x}{170-x} = .0211\ (t-61)$

Females, second cycle, $\log \frac{x-170}{226-x} = .0086\ (t-191)$.

It will be noticed that the calculation of the second cycle involves a change of the axes of the coordinates so that the new point of origin is near the point at which the first cycle of growth ended. The first cycle of growth in the female appears to terminate somewhat earlier than that in the male and the value of K, the constant, was greater in the growth curve of the female. These relations agree with the repeated observation that in early life the female grows more rapidly than the male. In the second cycle the female grows less rapidly than the male.

The close agreement between the observed and the cal-

[3] In Donaldson, *loc. cit.*

[4] Robertson, T. B., Univ. Calif. Publ. Physiol., 4: 211–228. 1915.

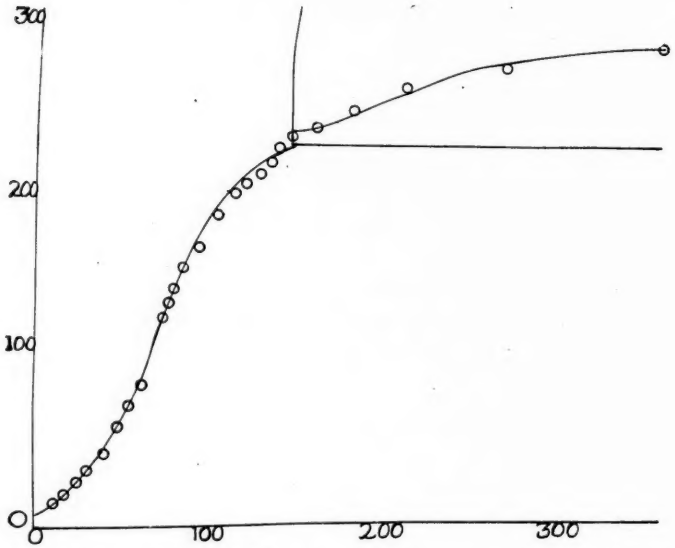

FIG. 1. Curve of growth for male white rat. . . . . , observed weight; ——, calculated weight. The weight for days 0–150 was calculated from log $[x/(228—x)] = .0187\ (t—73)$. The weight for days 150–365 was calculated from log $[(x—220)/(280—x)] = .0123\ (t—213)$.

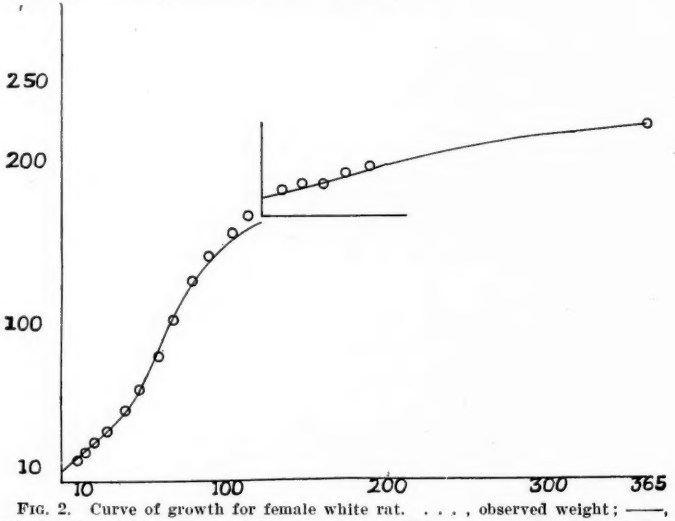

FIG. 2. Curve of growth for female white rat. . . . , observed weight; ——, calculated weight. The weight for days 0–124 was calculated from log $[x/(170—x)] = .0211\ (t—61)$. The weight for days 124–365 was calculated from log $[(x—166)/(226—x)] = .0086\ (t—191)$.

culated weights of the animals is shown by Table I and by Figs. 1 and 2.

B. The Growth of Rats Recovering from an Initial Period of Suppression

The published work of Osborne and Mendel contains very convincing evidence that the white rat possesses an inherent capacity for growth and that this capacity to grow survives long periods of suppression due to inadequate nutrition. Rats whose growth had been suppressed for over a year made prompt response and quickly reached mature size when the inhibiting factors were removed.

TABLE I

Growth of Albino Rats during their First Year [5]

Age (Days)	Males Weight		Age (Days)	Females (Unmated) Weight	
	Observed (Grams)	Calculated (Grams)		Observed (Grams)	Calculated (Grams)
First cycle			First cycle		
11	13	15	11	13	14
15	17	17	15	18	16
21	21	22	21	23	21
31	32	32	29	31	30
40	42	44	40	44	45
49	57	60	49	58	61
61	82	85	61	78	85
70	107	107	70	100	103
79	128	129	82	125	125
85	144	143	92	140	139
97	160	168	107	155	154
107	177	185	117	167	159
117	191	198	124	171	162
131	203	211	Second cycle		
143	218	217	124	171	179
150	225	220	131	179	180
Second cycle			138	182	182
150	225	229	143	183	183
157	227	230	150	185	184
164	231	232	164	185	188
171	236	234	178	192	192
178	239	236	192	196	196
185	240	239	365	226	224
216	253	251			
256	265	266			
365	279	279			

[5] Data from tables 3 and 64 in: Donaldson, H. H. "The Rat." Philadelphia, 1915.

A second feature of their results, which is no less noteworthy than the first, is the rapidity of growth after adequate diets were given. They show that the gains made by the rats whose growth had been previously suppressed were made in much less time than would be required for a rat on adequate diet to make the same gain in weight.[6] This inquiry is concerned only with certain characteristics of the rate of growth following the initial period of suppression and will not attempt any discussion of the nutritional aspects of the problem. The data discussed have been drawn from the work of Osborne and Mendel. It has not been possible to obtain records of enough individuals to give a statistically reliable average, yet the records employed are fortunately free from extreme fluctuations and are satisfactory as far as individual records can go.[7]

The first case to be discussed is that of a male rat (No. 1012) which at age 370 days had reached a body weight of 127 grams, having been fed alternately "gelatin food" and "milk food."[8] On the 368th day the ration was permanently changed to "milk food plus mixed food." This change in diet was promptly followed by rapid growth and the attainment of mature weight about 180 days later. It is evident from Osborne and Mendel's chart that the curve of "resumed growth" was steeper than the normal curve of growth. This difference is especially well marked during that portion of the time in which there is an actual increase in body size and less well marked during the time in which its increase is due to the formation and deposition of fat.

A quantitative study of the resumed growth of this animal shows the existence of two distinct cycles, each of which is expressed by an equation of the type already

[6] Osborne, T. B., and Mendel, L. B., *Amer. Jour. Physiol.*, **40**: 16–20, 1916.

[7] The writer is greatly indebted to Dr. Osborne and Professor Mendel not only for their kindness in furnishing data, but for their criticism of this manuscript.

[8] Osborne, T. B., and Mendel, L. B., *Jour. Biol. Chem.*, **18**: 95–107, 1914, Chart V.

discussed. From the time at which an adequate diet was supplied, until about the 432d day, the growth is expressed by the equation

$$\log \frac{x}{220-x} = .0193\ (t-363).$$

This indicates a cycle of growth which was completed at a body weight of 220 g. and which overlapped slightly

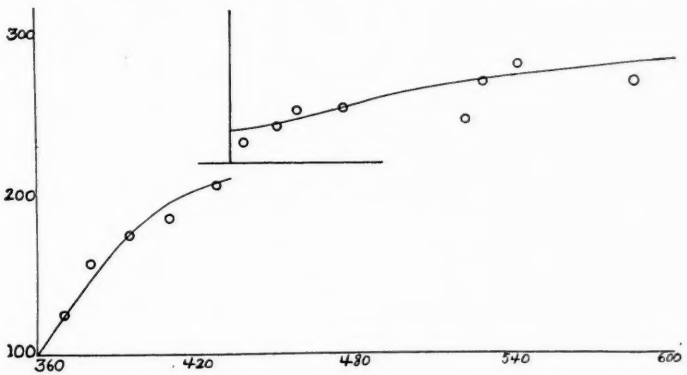

FIG. 3. Curve of growth of a male white rat following a suppression of 370 days. . . . . , observed weight; ——, calculated weight. The weight for days 360–432 was calculated from log $[x/(220-x)] = .0193\ (t-363)$. The weight for days 433–600 was calculated from log $[(x-220)/(288-x)] = .0091\ (t-475)$.

the second cycle of growth which was completed when a body weight of 288 g. had been attained. The equation representing the second cycle of growth is

$$\log \frac{x-220}{288-x} = .0091\ (t-475).$$

The second cycle may be represented by a curve having ordinate and abscissa axes originating at $y = 220$ and $x = 432$. The values are shown in Fig. 3. The agreement between calculated and observed values is as good as could be expected with only one animal in the sample.

The next case to be noted is that of a male rat (No. 2161) which was stunted by a diet of inadequate protein from age 38 days to age 248 days.[9] During this

[9] Osborne, T. B., and Mendel, L. B., *Jour. Biol. Chem.*, 23: 439–454, 1915.

period the increase in body weight was from 53 to 73 g. 220 days after an adequate diet had been begun the rat had grown to a weight of 300 g. The first cycle came to an end at the 368th day, when a weight of 230 g. had been reached, and is expressed by the equation

$$\log \frac{x}{236 - x} = .0180\ (t - 285).$$

The second cycle of growth is represented by the equation

$$\log \frac{x - 225}{305 - x} = .0150\ (t - 408).$$

For a graphic representation of these values see Fig. 4.

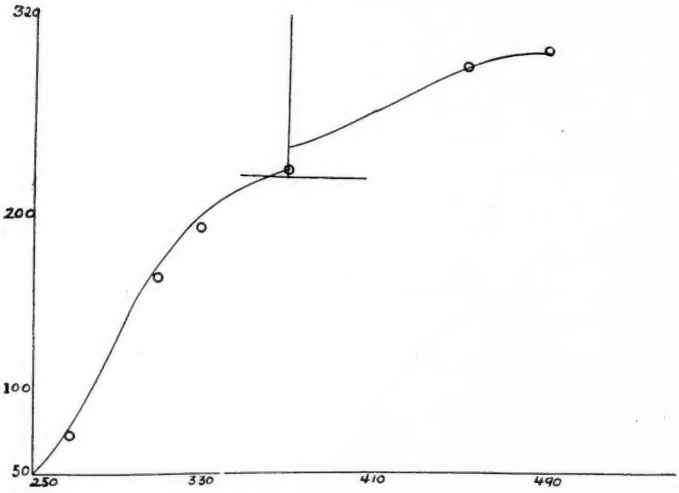

FIG. 4. Curve of growth of a male white rat following a suppression of 248 days. . . . . , observed weight; ——, calculated weight. The weight for days 248–368 was calculated from $\log [x/(236 - x)] = .0180\ (t - 285)$. The weight for days 368–488 was calculated from $\log [(x - 225)/(305 - x)] = .0150\ (t - 408)$.

The third case studied was that of a female white rat (No. 2033) whose growth had been retarded by limiting the daily quantity of food.[10] Although the weight of this animal was held below 60 g. for the remarkably long period of 510 days, it had not lost the capacity for growth.

[10] Osborne, T. B., and Mendel, L. B., *Jour. Biol. Chem.*, **23**: 439–454, 1915. Also *Amer. Jour. Physiol.*, **40**: 16–20, 1916.

After 135 days on an adequate diet it weighed 222 g.

The equations for the first and second cycles of growth of this animal are

$$\log \frac{x}{175-x} = .0260\ (t-519)$$

and

$$\log \frac{x-170}{230-x} = .0231\ (t-610).$$

For a graphic representation of these values see Fig. 5.

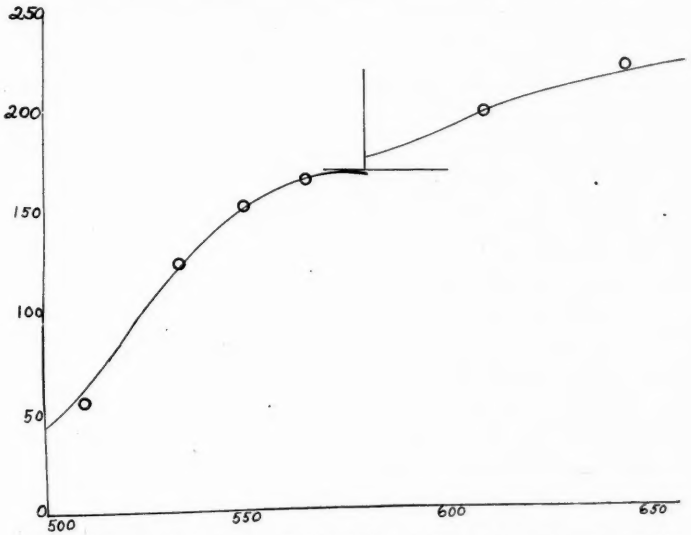

FIG. 5. Curve of growth of a female white rat following a suppression of 500 days. . . . . , observed weight; ——, calculated weight. The weight for days 500–580 was calculated from log $[x/(175-x)] = .0260\ (t-519)$. The weight for days 580–660 was calculated from log $[(x-170)/(230-x)] = .0231\ (t-610)$.

The constants of the different equations are grouped for convenience in Table II. From them it appears that male rats fed on adequate diet reached a weight of 280 g. at the end of their first year, and the female rats a weight of 230 g. in the same time. The females grow relatively faster in the first cycle than the males and in harmony with this property the values of K (the constant of the reaction) in the first cycles were greater than

those of the males. The values of K in the second cycles were less than those in the first cycles. In the case of males on adequate diet the value of K in the second cycle is about 66 per cent. of its value in the first, but in the females the value in the second cycle is only 33 per cent. of that in the first cycle.

The values of the constants are somewhat different in the cases of rats subjected to an initial period of suppression. Rat No. 1012 reached approximately the same body weight as a male rat fed continuously on adequate diet. Its weight was 127 g. when full feeding began on the 368th day; the gain was, therefore, 153 g. in 212 days. The male rats on adequate diet weighed 127 g. at the 77th day; their gain of 153 g. was, therefore, made in 286 days. The animal recovering from initial suppression, therefore, required considerably less time to make an increase from 127 g. to 280 g., than adequately fed animals required to reach the same stage of development.

The case of No. 2033 (female) has special interest because of the remarkably long period of stunting. The period of suppression started on the 39th day when the rat weighed 53 g. and ended on the 510th day when it weighed 57 g. The animal attained a weight of 222 g. in 135 days, following the resumption of full feeding. A female rat on adequate diet from the time of weaning would require 295 days to increase from 57 g. to 222 g., or more than twice the time to make the same gain. Other records of the time required by stunted and by adequately fed animals to attain a given weight are given by Osborne and Mendel[11] and in all cases the time was greatly reduced in the case of animals recovering from stunting. These authors likewise pointed out the broad biological significance of this faster growth rate. This question of rate is one of extreme interest in connection with the dynamical aspects of growth

[11] Osborne, T. B., and Mendel, L. B., *Amer. Jour. Physiol.*, 40: 16–20, 1916.

TABLE II

COMPARISON OF CONSTANTS

	First Cycle		Second Cycle		Duration of both Cycles
	a	K	a	K	
On adequate diet. Male	228	.0187	280	.0123	365 days
On adequate diet. Female	175	.0234	230	.0076	365 days
On restricted diets:					
No. 1012. Male	220	.0193	288	.0091	263 days
No. 2161. Male	236	.0180	300	.0150	286 days
No. 2033. Female	175	.0260	230	.0231	162 days

C. RATES OF GROWTH AS COMPUTED FROM VALUES OF dx/dt

It is unnecessary to dwell upon the prime importance of the study of rates in physiological investigations. We are concerned not only with what the organism is, but how it came to be what it is. As soon as we begin to study the problem of development, we encounter the question of rates. No better means of studying the rate of change in a system has yet been found than the differential calculus.

The differential equation representing the rate of autocatalysis is

$$\frac{dx}{dt} = kx\,(a-x).$$

a, x and t represent the same values as before, but $k = K/a$. We may proceed, therefore, to examine the derivatives of the equations used above to express the sizes of the animals at various time intervals. The values obtained for the growth of males and females are shown in Figs. 6 and 7 in comparison with the observed weekly increases of the animals which have been studied. The computed values were obtained from the tables published by Robertson[12] which give the values of $(1/Ka).(dx/dt)$ for corresponding values of $K(t-t_1)$.

The rate of growth of male rats on adequate diet was computed for each cycle from the figures in Table I. The

[12] Robertson, T. B., Univ. Calif. Publ. Physiol., 4: 211–228, 1915.

rates for each cycle are shown in Fig. 6. The growth rate at birth had an appreciable value, as one might expect.

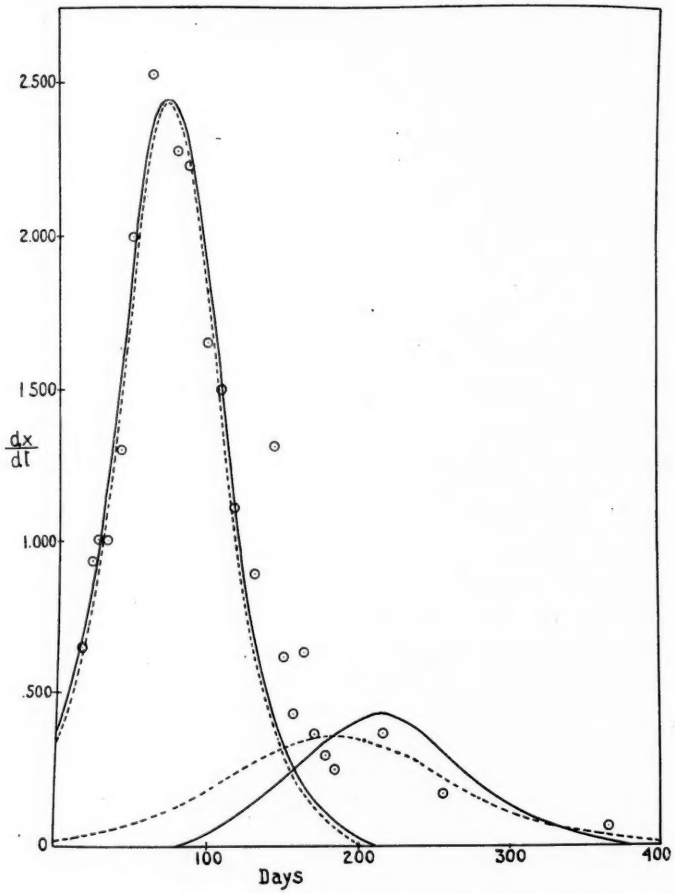

FIG. 6. Growth rates of male white rats. . . . . , observed increments of rats on adequate diet; ——, calculated values of dx/dt for rats on adequate diets; —— calculated values of dx/dt for rat recovering from a suppression of 370 days.

From birth to the 73d day the rate rose rapidly to its maximum, then fell to zero value of dx/dt at about the 215th day.

Before the first growth cycle was ended the second cycle had begun. The computed values show that this second cycle began about the 80th day of postnatal life, reached

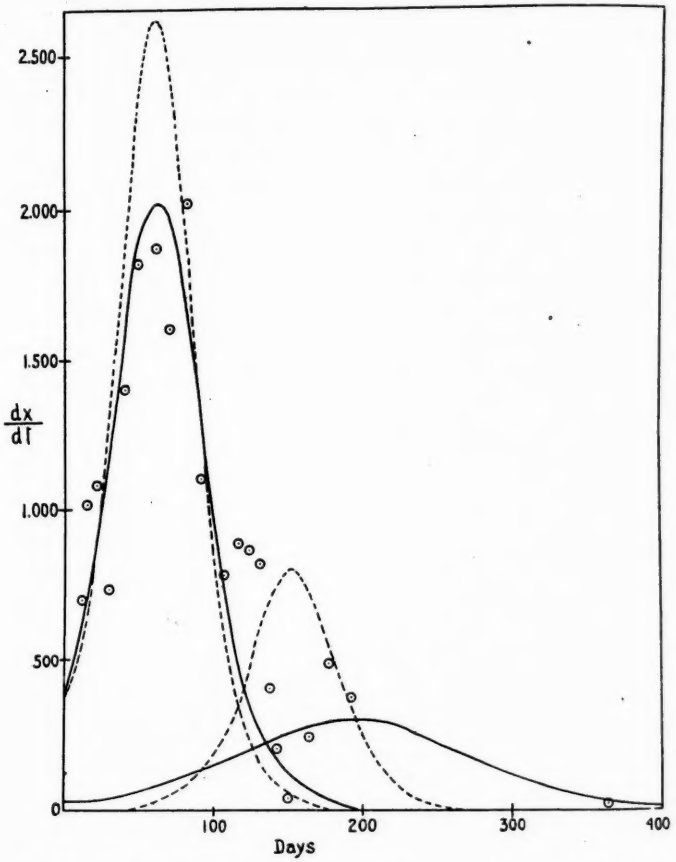

FIG. 7. Growth rates of female white rats. . . . . , observed increments of rats on adequate diet; ——, calculated values of dx/dt for rats on adequate diet; —— calculated values of dx/dt for rat recovering from a suppression of 500 days.

a maximum about the 213th day and ceased shortly before the 400th day. Since the value of $kx(a-x)$ approaches zero when x is very small and again when x is nearly

as large as a, it is possible to trace the curve in both directions until it reaches zero values of dx/dt. The actual values of dx/dt in the second cycle are much smaller than those in the first cycle.

Attention must be drawn to the way in which these two curves overlap. It will be noted that the second cycle of growth began shortly after the rate of the first cycle began to decline and that the first cycle continued to about the time of maximum rate in the second cycle. The true curve of the growth rate of these animals is, therefore, the arithmetical sum of the values of dx/dt for the various values of t. This is shown in Fig. 6 and agrees very well with the observed weekly increments.

The curves in Fig. 6 were obtained by plotting dx/dt as ordinate and t as abscissa.

If we let $dx/dt = z$, we may write

$$z - kx(a - x),$$

which when differentiated becomes

$$\frac{dz}{dx} = ak - 2kx.$$

If the right-hand member of this equation be equated to zero it will give the values of x for which z is either maximum or minimum.

Let $ak - 2kx = 0$; then

$$2kx = ak,$$
$$x = a/2.$$

Therefore the rate, z, is either maximum or minimum when $x = a/2$. To find whether z is maximum or minimum it is only necessary to get the second differential of the above equation

$$\frac{d^2z}{dx^2} = -2k.$$

Since this quantity has a negative sign z $(= dx/dt)$ is a maximum when $x = a/2$. In other words the rate of growth is a maximum when the cycle has reached a stage at which the weight of the animal is half the weight it attains at the end of that cycle. When $x < (a/2)$ the curve rises and when $x > (a/2)$ the curve falls.

The rate of growth of an animal recovering from an initial period of suppression may be studied in comparison with that of a rat on adequate diet. Take the case of rat No. 1012 (male). The computed values of dx/dt have been plotted in Fig. 6. The time at which dx/dt was a maximum in the first cycle was made to coincide with the maximum for the first cycle of the rats on adequate diet. This arrangement was adopted to facilitate comparison.

The curve for the first cycle as plotted in Fig. 6 is a very fair duplicate of the curve for the same cycle of growth for the rats on adequate diets. There is more difference in the case of the second cycle. The curve for rat No. 1012 has a maximum which is lower and occurs somewhat nearer the dx/dt axis than that of the other class of animals. In other words, the increased weight due to formation of fat in this animal began relatively earlier than in animals on adequate diets. In the main, however, there are no striking differences between the relative growth rates in the two cases, except that their maxima are nearer together.

The same sort of computations have been made for the growth of female rats and they are shown graphically in Fig. 7. An extended discussion of them is unnecessary, as it would be in many respects a mere repetition of what has been said. A comparison of the curves of female rats on adequate diet with those of rat No. 2033 shows (*a*) that the rate of resumed growth was faster in the second cycle, (*b*) that the second cycle was of shorter duration, and (*c*) that the maximum of the second cycle lay closer to that of the first cycle than in the case of rats on adequate diet.

In view of the fact that rats recovering from initial suppression reach mature weight more quickly than animals fed on adequate diets, it is somewhat surprising to find such a close similarity in the values of dx/dt for the same time intervals. One might expect that the curves for the recovering animals should be higher and steeper.

I believe that the reason for the quicker growth in the recovering animals lies, not in a faster growth rate in the cycle, but in the shorter time between the maxima of the two cycles. In other words, the final weight is reached more quickly because the second cycle of growth commences relatively earlier and is added to the first cycle. Unfortunately, the weights of recovering animals were not taken at sufficiently frequent intervals to afford data upon the actual rates of growth in this class.

If we assume that growth is the result of a catalyst acting upon a substrate, it seems that we have a key to the explanation of what is observed. The catalyst of the first cycle was produced in the pre-natal stages. Although there was no appropriate substrate available in the starved animals, the catalyst did not disappear. When an appropriate substrate was given, this catalyst acted upon it, producing a cycle of growth essentially equivalent to that shown by animals fed on adequate diets. This may mean that the catalyst persisted unimpaired until it was destroyed in the course of the reaction. The catalyst responsible for the second cycle likewise appeared and induced the formation of fat. If the second catalyst is in some way dependent upon a time factor for its formation (or activation) it is plain that it should show its activity relatively earlier in the case of animals recovering from a long period of initial suppression, because of a *quasi* cumulative age effect. The effect of this would be what we have seen to happen, viz., a crowding of the cycles nearer together.

The writer is fully aware of the hazards encountered in attempting to represent so complex a reaction as growth by a simple formula. The phenomena of growth appear, however, to be coordinated into a single self-consistent process, in which many chemical and physical factors are combined. The possibility of expressing growth by a simple formula showing that an increase in mass is definitely related with a function of time ought to lead to considerations of a fundamental nature.

D. Summary

1. The growth of white rats during the first year shows two cycles, and each cycle follows the course of an autocatalytic reaction. The first cycle covers the period in which the skeleton rapidly increases in size; the second covers the period in which there is a production and deposition of fat.

2. The equations for the growth of the two sexes differ in their constants, but each expresses the course of an autocatalytic reaction.

3. The earlier period of stunting did not prevent the animals from attaining the full weight characteristic of their sex after having an adequate diet. An equivalent gain in weight was made more quickly in the animals recovering from suppression than in animals on adequate diet.

In other words the animals grew somewhat more rapidly during their period of recovery.

4. The growth of white rats recovering from a long period of suppression follows the curve of autocatalysis, though a portion of the first cycle has been run during the long period of suppression.

5. The differential equations expressing the growth rates show that the two cycles overlap to some extent. The sums of the overlapping values approximate closely the observed increments. The second cycle of growth of rats recovering from starvation began and reached its maximum relatively earlier than in the case of rats on adequate diet.

6. There is evidence for the idea that each cycle of growth in this case had its specific catalyst and that the potential activity of the catalyst was not impaired by long periods of inadequate nutrition.

SHORTER ARTICLES AND DISCUSSION

ESTIMATING THE NUMBER OF GENETIC FACTORS CONCERNED IN BLENDING INHERITANCE

IN *Science* for July 29, 1921, Dr. W. E. Castle develops a "method of estimating the number of genetic factors concerned in cases of blending inheritance," which appears so simple, so attractively cogent, and so usable, that it is to be feared that the erroneous assumptions upon which it is based may be given less consideration than they merit.

About seven years ago,[1] I took some pains to point out certain fallacies which had crept into genetical literature through the tacit (or express) assumption that the several factors affecting the size of an organism or its parts, or the intensity of development of any character, are similar to each other in kind and equal in effectiveness. It has seemed to me that since that time there has been marked improvement in the literature dealing with this particular phase of genetical phenomena—whether in response to my paper or through independent following out of the simple logic of the case, it matters not. It comes as a distinct shock, therefore, to see the sudden reversion in Dr. Castle's paper to a supposedly outgrown and abandoned conception.

Dr. Castle has probably forgotten the bearing of my paper, though he commended it very highly in a letter written at the time of its publication; for so far as I can now recall he has never referred to it in any of his frequent papers, published since that time, on subjects involving the multiple factor hypothesis as an explanation of blended inheritance and the modification of a so-called "unit-character." This omission has been the more interesting because my paper even gave the precise interpretation of his hooded-rat case, which has been very lately espoused by him.[2]

Referring to the particular question now under discussion, my paper said:

> Attempts to determine how many plural determiners for any quantitative character are involved in a particular cross are as yet premature. Such attempts are based on the unproven hypothesis

[1] "Duplicate Genes for Capsule-form in *Bursa bursa-pastoris*," *Zeitschr. f. indukt. Abstamm. u. Vererb.*, **12**: 97–149. 1914.

[2] Castle, W. E., "Piebald Rats and the Theory of Genes," *Proc. Nation. Acad. Sci.* [*U. S. Amer.*], **5**: 126–130, 1 fig., April, 1919.

that the range of variability in F_2 equals the combined ranges of P_1 and F_1 generations, and the unwarranted assumption that the different plural determiners are essentially equal in effect.

What was premature seven years ago might be conceivably no longer premature, of course, but since the basis upon which Dr. Castle now proposes to estimate the number of such plural factors in specific crosses involves the same fallacies which made previous attempts untenable, it is needful to reiterate that nothing in the evidence justifies the belief that this new plan will give any sort of approximation to the actual facts. Nevertheless, the method proposed by Castle may be expected to have a certain amount of interest as representing a limiting case.

It is absurd to suppose that the height of a man will be as much affected, severally, by the factors which increase the thickness of the scalp, as by those which affect the length of the long bones of the legs; or that factors which produce changes in the number of internodes of a plant will generally add severally the same increment to the stature of the plant as will factors which increase the length of some or all of the internodes. Castle recognizes this weakness, but seeks to minimize its importance and declares that "no other assumption will permit of a general treatment of blending inheritance." He means, of course, merely that on no other basis can a generalized mathematical scheme be developed such as that which he has here presented.

But even if we allow such assumption of equivalence of genetic factors to pass on the ground of mathematical expediency, there are several other conditions involved in Castle's scheme which are equally unwarranted and which will profoundly affect the validity of the conclusions arrived at.

For example, the two strains mated together are supposed to stand at the two extremes of the total potential genetic variability in their progeny and all of the determiners are assumed to be additive in their effect, so that if we let the factors be represented in the usual manner by letters, the lesser parent must have the formula *XXaabbccddeeff* . . . , while the larger parent occupies the other extreme *XXAABBCCDDEEFF* . . . . It need scarcely be pointed out that while such a situation might be realized in some specific case it could not be generally true, and the greater the number of factors involved in any specific cross, the less likely would it be that the larger parent would possess them all and the lesser parent none. In my paper, referred to above, I said in regard to this point (p. 132):

Nilsson-Ehle (1911) has described a case in which the range of variation in the length of heads of wheat in the F_2 considerably exceeded the combined ranges of the two parents. Hayes (1912) has found a similar case in the number of leaves in tobacco, and Emerson and East (1913) have seen the same phenomenon in the length of internode and total length of stalks in maize. It seems probable that such transgressive variation may be the rule rather than the exception when very complex characters are investigated; for it is hardly to be expected that a large number of plural determiners affecting such a character shall all act in the same direction or that the parent having the highest development of the given character shall generally contain all the genes which the other chosen parent possesses. Whenever such transgressive variability is producible by the genotypic recombinations of parental characters, the frequency with which F_2 individuals simulate either parent gives no clue to the total number of plural determiners which have been brought together, with respect to any character under consideration.

We might even derive a mathematical expression for the probability that the parents would stand at the extremes of total genetic variability, by assuming that the two parental types are taken at random. This would be perhaps a fair assumption, since the number of factors can not be determined by inspection. There would then be, if we let n represent the number of factors involved in the cross, $n!/2$ ways in which the event in question can not happen and only one way in which it can happen; hence the probability that all the factors would be present in the larger parent and absent in the lesser parent would be as 1 to $n!/2$. In the specific case of East and Emerson's corn, cited by Castle as having about 15 factorial differences, ($n=15$), there would be, therefore, 633,477,184,000 chances to one against all of these size-modifying factors being present in the larger parent and absent in the smaller parent. When we let $n=50$ or 150, to agree with the numbers indicated for the rabbit crosses, the chances become practically infinitesimal and we must fairly conclude that it has never happened, and never will happen, that a cross involving so many independent size differences has been made, or will be made, between individuals standing at the opposite extremes of the total potential genetic variability.

The remark made in my paper on duplicate genes, regarding the inadequacy of the frequency with which parental types are duplicated in the F_2 as an indication of the number of factors involved, applies equally well to the validity of conclusions drawn from changes in the F_2 standard deviations. The

correctness of this conclusion will be made sufficiently obvious by consideration of a simple illustrative case: Let us assume that there are involved in a given pair-mating the six duplicate size-factors, ***AABBCCDDEEFF***, and their absences or corresponding recessives, *aabbccddeeff*. The relation of the F_1 and F_2 variability will be exactly the same no matter which of the four following types of mating is made: (*a*) *aabbccddeeff* × ***AABBCCDDEEFF*** (difference between parents, 12 units); (*b*) ***AA***bbccddeeff × *aa*BBCCDDEEFF (parental difference, 8 units); (*c*) ***AABB***ccddeeff × *aabb*CCDDEEFF (parental difference, 4 units) or (*d*) ***AABBCC***ddeeff × *aabbcc*DDEEFF (parental difference, zero). Neither Castle's original scheme nor Wright's suggested modification[3] of it can be true, at one and the same time, for more than one of these types of mating and they have been specifically designed only for the first-mentioned type (*a*); but as we have seen in a previous paragraph, if the number of factors involved is large, the number of matings of type (*a*) is almost infinitesimal in comparison with the number of matings of the other types, (*b*) + (*c*) + (*d*) + (*e*) + . . . to *n* terms.

It is not necessary, however, to suppose that all factors which affect a blending character are additive. Indeed, it is quite certain that they are not, and that some factors act in a negative direction and others in a positive direction. Inhibiting and depressing factors have been fully demonstrated, as may be exemplified by such classic cases as the inhibitors of horn production in cattle and sheep; the inhibitor of indeterminate growth in the tail of the Japanese long-tailed fowl; the condensation factor characteristic of the *compactum* type of wheat; and a series of dominant depressing factors which Davenport's[4] studies have made probable in the case of human statures. The fact that Castle himself was able to make progress in the minus direction in his selection experiments suggests the probable accumulation of factors acting in a negative direction, though in this case the evidence is not decisive, as the same effect would have been secured by the gradual elimination of factors acting in a positive direction. It seems to me that the combined action of plus-act-

[3] Castle, W. E., "An Improved Method of Estimating the Number of Genetic Factors Concerned in Cases of Blending Inheritance," *Science*, 44: 223, Sept. 9, 1921.

[4] Davenport, C. B., "Inheritance of Stature," *Genetics*, 2: 313–389. July, 1917.

ing and minus-acting growth factors gives a very true, though somewhat formal conception of the general situation in all organized beings;—the interplay of growth-promoting and growth-inhibiting factors may be thought of, in a figurative sense, as forming, within the limits of fluctuating variation, a sort of elastic "mold" into which any organism, whether plant or animal, develops, and which gives it its wonderful specificity of form and size.

But on the basis of this conception of factors, acting, some in a positive and some in a negative direction, the combined action of the negative group exactly balancing the combined action of the positive group, and jointly determining the mean size or the average condition with respect to any blending character which may be under consideration, it becomes unnecessary to assume the absence of dominance. I have been teaching my students for the past six years that the postulation of lack of dominance which has always been made the basis of the multiple-factor interpretation of inheritance of size or of other blending characters is wholly unnecessary and that those who have discussed this type of inheritance have been led to place an altogether unnatural and unwarranted stress on the occasional occurrence of incomplete dominance in other cases.

But whether any or all of the size-factors are dominant or not materially affects the amount of change which they effect in the value of the F_2 standard deviation, and must correspondingly change the estimate of the number of factors involved when that estimate is based on the value of these F_2 standard deviations. As a simple example, I may cite the hypothetical illustration given in my 1914 paper (p. 129), referred to above:

> Thus, if a plant possessing a partial inhibitor or reducer of internode-number be crossed with another plant having a stimulator for internode length, all the other genes being the same in the two cases, the height of the F_1 plants would be intermediate between the heights of the parents, with variability due alone to fluctuation, as it is in the homozygous parents. The F_2 would show increased variability, and *this increase would appear greater if the two differentiating genes were dominant, than if dominance were absent.*[5]

To illustrate this fact further, let us assume that the six size-modifying factors which differentiate two mates are ***AABBCC***, acting in the plus-direction, and ***DDEEFF***, acting

[5] Not italicized in the original.

in the minus-direction, and that the effect of each factor pair is represented by 2 units, thus making up the total of 12 units arbitrarily chosen by Castle. If the various permutations are worked out, the F_2 series of frequencies is found to be as follows:

TABLE I

DISTRIBUTION INTO SIZE-CLASSES RESULTING FROM THE INTERPLAY OF SIX FACTORS, 3 POSITIVE AND 3 NEGATIVE, WHEN DOMINANCE IS WANTING AND WHEN DOMINANCE IS COMPLETE.

Condition as to Dominance	Class Values and Frequencies												
	−6	−5	−4	−3	−2	−1	0	1	2	3	4	5	6
No dominance	1	12	66	220	495	792	924	792	495	220	66	12	1
Dominance complete	27		270		981		1,540		981		270		27

These frequencies are exhibited graphically in Fig. 1, the curve produced by dominant factors being reduced to the same area as the curve of no dominance by dividing each frequency by 2. It is thus seen clearly that whether dominance is present or absent, the resulting curve is of the same general type.

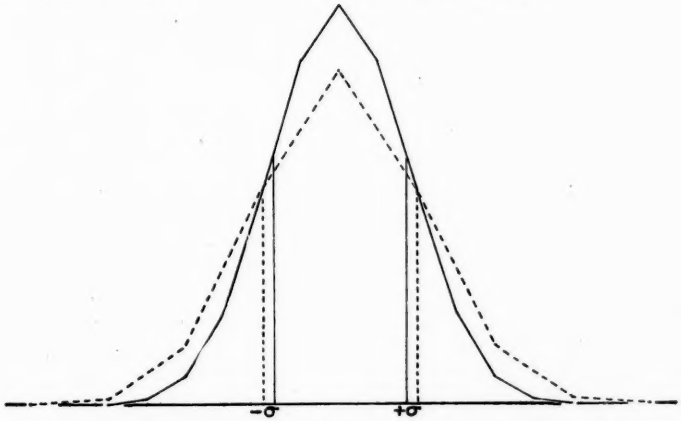

FIG. 1. The variation produced by six equivalent factors, three acting in plus direction and three in minus direction. The unbroken curve shows the result when dominance is wanting. Dotted curve shows the effect of complete dominance in all six factors.

The standard deviation of the curve with no dominance is $\sqrt{3} = 14.43$ per cent., as Castle has stated, but when all of the

six factors are completely dominant the standard deviation is $\sqrt{4.5}$, or 17.67 per cent., which is the percentage corresponding to 4 factors as given in Castle's Table II. In other words, six factors with dominance produce as great an increase in the F_2 variability as four factors would produce if none of them exhibited dominance.

It can be shown in the same way, by means of a simple test case, that Castle is entirely in error in saying that "if one factor really has an influence greatly superior to that of other factors in a case of blending inheritance, this will be seen in the production of asymmetrical or multimodal variation polygons in F_1 and F_2." The consequence he mentions would follow only in case the factor having the greater influence happened to be dominant, for in the absence of dominance each factor enters into combination with every other factor in a 1 : 2 : 1 series of intensities which gives the probable-error type of distributions. Thus, if we assume that four factor pairs *AABBCCDD* differentiate two chosen mates with respect to some blending character, and that three of these factor pairs, *AABBCC*, each acts in a plus direction with a value of 2 units, and the fourth factor pair, *DD*, acts in a minus direction with a value of 6 units, the F_1 will be intermediate as before while F_2 will give the series of frequencies shown in Table II.

TABLE II

DISTRIBUTION INTO SIZE-CLASSES RESULTING FROM THE INTERACTION OF THREE FACTOR PAIRS ACTING IN THE PLUS DIRECTION, EACH ADDING TWO UNITS, AND A FOURTH FACTOR PAIR ACTING IN THE MINUS DIRECTION AND SUBTRACTING 6 UNITS

Condition as to Dominance	Class Values and Frequencies												
	−6	−5	−4	−3	−2	−1	0	1	2	3	4	5	6
No dominance	1	6	15	22	27	36	42	36	27	22	15	6	1
Dominance complete	3		27		81		82		9		27		27

It will be noted that while the curve of no dominance in this case is not quite a typical probable-error curve, it is nevertheless perfectly symmetrical and the deviations from the typical curve are such that they would never be detected when the several classes are modified by concurrent fluctuations from environmental causes.

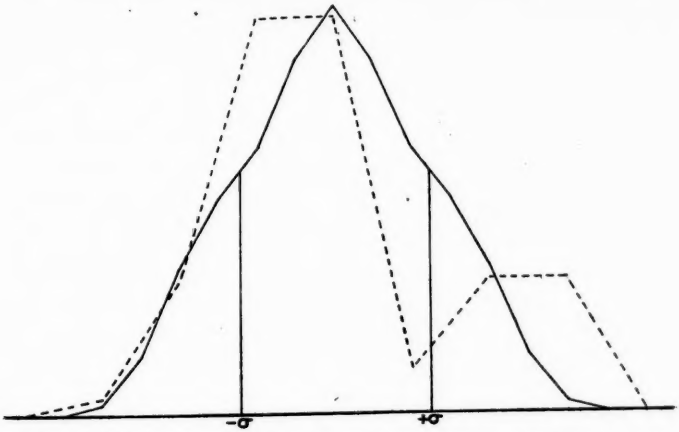

FIG. 2. Variation curve for four factors, three acting in plus direction, each with an effect of two units, and one acting in minus direction, with an effect equal to six units. Unbroken curve shows no dominance in any factor; dotted curve shows effect of complete dominance in all four factors.

The standard deviation of this series of values is $\sqrt{6}$, or the same as would be produced by 3 factors if all were of equal value, as shown in Castle's table. This curve is shown graphically in Fig. 2 together with the corresponding curve for factors weighted in the same manner, but exhibiting dominance. Such an irregular, multimodal curve as the latter would be easily detected if there were no fluctuating variations, but Castle apparently overlooks the effectiveness of fluctuating variations in obscuring the details of the underlying curve of genotypic variability. When he interpolates between the standard sizes of two breeds of rabbits 50 to 150 centers of genetic stability, he should take into account that the fluctuations about each of these centers will be sufficient to scatter the individuals which belong in any one class over quite a considerable number of other classes, thus filling up the gaps and hiding a great deal of putative multimodality in the genotypic curve, which would result from inequalities in the relative effectiveness of the several plural factors involved in any case of blending inheritance. Castle apparently thinks that this confusing concurrence of genetic and fluctuating variation could be dissolved to a certain extent by rearing "adequate numbers" in F_1 and F_2, but it must not be forgotten that the masking effect of fluctuations advances *pari passu* with the increase in the size of the population. Only the irregularities due

to too small size of the random sample could be eliminated by the rearing of larger numbers; the effects of the interplay of numerous environmental factors could not be thus eliminated.

From the foregoing considerations it must be clear that Castle is altogether too sanguine as to the value of his method when he says:

> It is perhaps not to be expected that results more than approximately correct would be given by this method, *unless fairly large numbers of both F_1 and F_2 individuals have been studied.*[6]

I believe the conclusion is justified that even a "fairly large" number of individuals of the F_1 and F_2 could not be expected to give correct estimates of the number of factors interacting in any case. As between the method of Castle in estimating the number of hypothetical duplicate factors operating in any case on the basis of the change they produce in F_2 standard deviations, and the method of Punnett, of formulating a genotypic situation on the basis of a small number of definitely weighted factors, I am convinced that the latter method is much to be preferred, even though it does not lend itself readily to a "general treatment of blending inheritance."

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DR. SHULL has kindly sent me his manuscript in advance of its publication and generously asks me to comment on it, an invitation which I gladly accept.

The difficulties which he thinks might be encountered in applying the method, which I have suggested, for estimating the number of genetic factors involved in cases of blending inheritance, seem to be essentially these.

1. The possible unequal influence of the several factors which are responsible for a case of blending inheritance, making it difficult to estimate their number from the total effect observed.

2. The possibility that some of the factors may be dominant in character and others not.

3. The possibility that some may be positive in action and others negative or inhibitive.

I think that these difficulties arise chiefly from the attempt of Dr. Shull to extend the application of the method beyond the field for which it was proposed. Cases of dominance or of trans-

[6] Italics are mine.

gressive variation in F_2 would not come within the scope of blending inheritance as I defined it, when proposing the method, but only cases in which both F_1 and F_2 are intermediate between the parent races. But Dr. Shull maintains that some dominant *factors* may be involved even where dominant characters are not in evidence. This may be admitted as a possibility even though we have no evidence for it. What of it? Dr. Shull formulates a test case, the strongest one imaginable, in which *all* (six) factors affecting a size character are "completely dominant" and finds that in this case the method which I suggested would indicate a smaller number of factors than the true one, or four instead of six. If this is the maximum error to be anticipated when *all* factors are completely dominant, and it is really doubtful whether *any* factors are dominant in the case of blending characters, the possibility need not give us great concern. Further, if all factors involved in producing a character are "completely dominant," how can the character itself keep from being dominant? And if it is dominant, the case will be automatically removed from the field of blending inheritance. Shull seeks to avoid the difficulty in his hypothetical case by putting three dominant factors in one parent race and three in the other, but this arrangement, by recombination of factors, in F_2 would result in segregation of the parental types or in transgressive variation, either of which events would remove the case from the category of blending inheritance as I have defined it.

The supposed difficulty, that some factors involved in the production of blending characters may be positive in action while others are negative, is purely formal. With three positive and three negative factors, in his hypothetical case, Shull comes out with identically the same distribution in thirteen size classes that I calculated for the same number of factors all positive, both of us assuming no dominance to occur.

It remains to deal with the first-mentioned difficulty, the possible unequal influence of the several factors assumed to occur in blending inheritance. I had anticipated this difficulty in my first paper, but had assumed that, "if one factor really has an influence greatly superior to that of other factors in a case of blending inheritance, this will be seen in the production of asymmetrical or multi-modal variation polygons in F_1 and F_2."[1] Shull challenges this statement and artfully constructs a case to

[1] I should have limited the statement to F_2.

disprove it in which the influence of one factor exactly equals and negatives that of three other factors. In this case he finds that the variation curve is symmetrical when no dominance occurs, but asymmetrical and bimodal otherwise. Had the hypothetical factors been less carefully weighted by Shull, he would not so easily produce a symmetrical F_2 curve. Consider an actual case in which a single factor of superior influence occurs and yet in which there is no dominance, that of the blue Andalusian fowl. Black mated with splashed white produces blue in F_1, an apparent blending. Yet F_2 falls, as every one admits, into three distinct classes notwithstanding the occurrence of one or more modifying or inhibiting factors affecting the result in a minor degree (Lippincott). "Fluctuating variation" does not here obscure segregation, as Shull assumes would be true in hypothetical cases of blending inheritance in which factors of very unequal influence occur, even when large numbers are studied. Now I should not class the case of the Andalusian fowl as blending inheritance, but I think it may serve to show that Shull's objection is not well founded, in accordance with which he assumes that a factor of major influence will not be readily detected, even when it is operating in conjunction with minor modifying factors.

W. E. CASTLE

TO THE EDITOR OF THE AMERICAN NATURALIST: The foregoing article and rejoinder are submitted for publication in their original form. I do not think that Castle's "comment" adequately meets the several difficulties which I have pointed out, and he presents no considerations which seem to me to warrant a modification of the statements I have made. Others who are interested in the topic under discussion may be depended upon to recognize the validity or non-validity of any of the propositions made by either of us. It should be said, however, that there was nothing "artful" in my choice of an illustrative case to show that inequalities in the effectiveness of the several genes do not necessarily produce asymmetrical and multi-modal variation-polygons when dominance is not present. The case could have been made still more striking by using a larger number of factors, but the additional labor required did not seem necessary. Obviously Castle has not tried out cases in which the factors are weighted differently from the weightings I assigned to them,

or he would not say that in that case I "would not so easily produce a symmetrical F_2 curve."

That he has not assimilated the significance of the inter-play of plus-acting and minus-acting factors is shown by his question:

> If all factors involved in producing a character are "completely dominant," how can the character itself keep from being completely dominant?

The curves in my Fig. 1 are an adequate answer to this question.

Finally, it seems hardly necessary to point out the inadequacy of the case of the Blue Andalusian fowl, as a proof that inequalities in genotypic variation are not masked by fluctations when the amount of fluctuating variation is large in comparison with the distances between the centers of genetic stability which are determined by the numerous factor combinations putatively involved in the several examples cited in Castle's original paper.

GEO. H. SHULL

GENETIC TERMINOLOGY

THE genetic terms recently proposed by G. H. Shull[1] seem to supply a real need. Their general use would certainly tend to reduce both the danger of ambiguity and the need for cumbersome descriptive phrases. Probably a few additional numerical terms, such as *dizygous* and *trizygous*,[2] would also be useful. Some existing equivalents have an obvious disadvantage with respect to compounding; the compound *trichromosomal*, for example, could not replace *trizygous* ("dependent on three *pairs* of chromosomes"). *Monozygous* and *pleiozygous*, although often interchangeable with *linked* and *unlinked*, should be useful; the latter, for example, to characterize genes that are located in several pairs of chromosomes but are not all necessarily unlinked with each other. These words are so interrelated among themselves, and so closely related to terms in general use, that all their advantages can be realized with little effort.

Shull suggests that it is time "to abandon the use of 'Mendelian' and 'non-Mendelian' as definite categories, and to adopt

[1] Shull, George H., "Mendelian or Non-Mendelian?" *Science*, N. S., 54: 213–216. Sept. 9, 1921.

[2] For the Greek numeral prefixes see Blakeslee, Albert F., "Types of Mutations and their Possible Significance in Evolution," AM. NATURALIST, 55: 254–267. 1921.

other terms which will have greater precision of meaning." Let us accept his timely proposal, which obviously applies especially to the more technical and precise terminology of genetics. With his new terms available, we may safely relegate the older ones, aside from historical references, to the more popular language of science.

Shull uses the older words to illustrate the application of his proposed terminology, but he does not specifically discuss their future delimitation in case they still retain a certain usefulness. Their future, I believe, deserves consideration. It seems certain that they will remain familiar words because of their historical value, in relation both to Mendel's work and to its earlier extension. Doubtless they will long be especially useful in the more popular presentation of genetic topics, to obviate burdensome use of more precise but more formidable expressions.

Historically, it is plain that the meaning of *Mendelian* has very largely kept pace with the widening conception of the fundamental applicability of Mendel's theory, although often, as Shull states, with the addition of qualifying expressions. When this widening process reaches the farthest point of practical usefulness, it leads to a broad definition of *Mendelism* which, I believe, deserves general acceptance.[3] It furnishes, for example, a convenient and familiar popular equivalent of *zeuxis* for the characterization of "chromosomal heredity," at least so far as the inheritance phenomena of sexual reproduction are concerned. All other senses of *Mendelian* seem to require more technical detail in definition, or to be otherwise less useful for the purpose in question.

Again, the most significant conflicts of "Mendelism" with its critics have raged along a line of demarkation essentially corresponding to the broader definition. "Mendelians" once encountered frequent denials of the completeness and the generality of segregation, and frequent assertions that new somatic ratios implied other modes of inheritance of equal significance with Mendel's. The triumph of the chromosome theory has been the definitive establishment of the fundamental significance of "Mendelian heredity."

Finally, the broadest definition is fully justified logically, although it may not be superior, in this respect to some other delimitations of the term. It may be held with good reason that

[3] Not forgetting, of course, that the older usage varies.

even linkage represents an *addition* to Mendel's genetic theory, rather than an *exception* to it. His scheme of independent separation and recombination of potentialities at gametogenesis is still adequate for the innumerable cases resembling his. Further, all genetic factors belong to theory rather than to observed fact, as do atoms and molecules. A gene is a *supposed* reality; it is something which many geneticists now *assume*, on the basis of evidence which they consider essentially conclusive, to be an *actual* part of a chromosome. The idea of lethal genes, therefore, or even that of the gene as a part of a chromosome, just as truly constitutes an addition to Mendel's genetic theory as a whole, as does the explanation of linkage ratios. The difference is, from this viewpoint, one of degree rather than of kind. If we admit some added hypotheses as Mendelian, why should we necessarily exclude any others which plainly relate to the same unified nuclear mechanism?

Even if we hold, as we may, that Mendel's theory has been revised as well as extended, its most fundamental feature, by present standards, is left unchanged. What, from our present viewpoint, is Mendel's most fundamental genetic conception? Is it not that of a *genetic shuffling*, a segregation and recombination, of genetic units which maintain their individuality throughout the processes of reproduction and of development?[4] Most usefully and even most commonly, it seems to me, *Mendelism* signifies *the general type or mode of inheritance* whose most fundamental principle of character distribution was discovered by Mendel; and this is "zeuxis," or chromosomal heredity, in sexual reproduction. This delimitation of *Mendelism* seems to me fully as good logically, better in accord with history, and much more promising of future usefulness in the field where the term is still needed, than any of the less inclusive senses in which it has been employed.

Dr. Shull says in correspondence, "I can see no objection to the *general non-technical use* of the words 'Mendelian' and 'Mendelism' in just the sense which you propose." And I believe that Morgan, East, Jones and Wright are far from being alone when they positively favor the broader definition.

[4] Bateson, W., "Mendel's Principles of Heredity." 1909. Cambridge Univ. Press. (See p. 13.)

Morgan, T. H., Sturtevant, A. H., Muller, H. J., and Bridges, C. B., "The Mechanism of Mendelian Heredity." 1915. New York, Henry Holt & Co. (See p. 1.)

Let us, then, take the course which is obviously more useful, and also honor the memory of the great pioneer of genetics, by applying his name to his great idea in all its later ramifications. But—wherever newer and more precise terms will better promote the science of genetics, let us be ready to use them. Shull's recent contribution to genetic terminology promises considerable and lasting usefulness.

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IN connection with Dr. Shull's[1] interesting and important proposals concerning genetic nomenclature, attention should be called to a situation which neither these proposals nor the terminology in general use recognize. It is customary to refer to individuals carrying single X or Z chromosomes, as being heterozygous for sex-linked genes. For some time this has seemed to be ill-advised to the writer.

The situation prevailing in an XX or ZZ individual heterozygous for a sex-linked gene clearly differs from that of an XY (or XO), or a ZW individual in the vast majority of cases, though Schmidt's work on *Lebistes reticulatus*, to which Dr. Castle[2] recently called the attention of American workers, possibly indicates that for XY individuals it does not necessarily always differ. In the one case, usually there is a demonstrable allelomorph, not infrequently competitive enough in its expression to produce more or less of an intermediacy between the two homozygous forms. In the other, usually there is not.

The term heterozygous, as much as homozygous, indicates an allelomorphic pair, yet in XY and ZW individuals, with the one possible exception noted, a pair of sex-linked genes has not been demonstrated, and is clearly impossible for XO individuals. To all appearances the sex-linked genes in such individuals are without synaptic mates. They are therefore simplex but not heterozygous.

In order to recognize this situation, and in a measure describe it without using presence and absence terminology, and in harmony with the terms proposed by Dr. Shull, I should like to suggest the noun *hemizeuxis* (a half yoking) and the corresponding adjective *hemizygous* (half yoked). Should such

[1] Shull, Geo. H., 1921, *Science*, N. S., 54: 213–216.

[2] Castle, W. E., 1921, *Science*, N. S., 53: 339–342.

a suggestion prove acceptable there would be the three adjective series: homozygous, heterozygous, and hemizygous, referring to the three possible conditions with respect to any single gene, namely, "like mates," "differing mates," and "no mate."

The term might also be used in cases where a non-deficient chromosome is paired with a deficient one. The deficient individual would be hemizygous for the genes at those loci of the non-deficient chromosome which were involved in the deficiency of its mate.

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CROSS-OVER VALUES IN THE FRUIT-FLY, DROSOPHILA AMPELOPHILA, WHEN THE LINKED FACTORS ENTER IN DIFFERENT WAYS

THE factors for bar, round, red and white eye are sex-linked, being located in the X-chromosome, as shown in inheritance. For example, when a bar-eyed male is mated to a normal female all the resulting male offspring (F1) are normal and all of the females bar eyed, as bar is dominant; that is, factors that occur in the X or first chromosome have a " criss cross " mode of inheritance following the distribution of the X-chromosomes, there being but one X-chromosome in the males and two in the females.

The relative positions of the linear series of factors in the X-chromosome of *Drosophila* have been determined by Morgan and Bridges (237 Carnegie Institution). Factors that lie near together in the chromosome are more likely to be transmitted in the same combinations to the gametes than those that lie far apart; that is, the strength of linkage depends on the distance apart of the factors. The failure to transmit the same combinations of factors that enter from the parents to all the offspring is due to a crossing-over of some of the factors. For example, a red bar-eyed fly, mated to a white round-eyed fly, give in the second generation (F2) white bar and red round-eyed flies, as well as flies like the original parents; that is, there has occurred a recombination of the factors due to crossing-over.

Are the cross-over values the same when the linked factors enter in different ways? My experiments performed in the University of Chicago Laboratories give data relative to this question. Using the presence and absence hypothesis, let (B) represent the factor for bar and (R) the factor for red, then let

(b) represent the absence of the factor for bar and (r) the absence of the factor for producing red-eyed flies. The factorial composition, then, of the red bar-eyed flies is (BR) and for white round-eyed flies (br). As both the factors for bar- and red-eyed flies are sex linked—that is, they occur in the X-chromosome, there being but one in the male and two in the female, as above mentioned. Then the male red bar-eyed flies are represented by (BR) and the female white round-eyed flies by (br—br).

Matings were made as follows:

(2 pairs) Red bar-eyed males (BR). White eyed females (br—br) F1 171 White round-eyed males (br). 184 Red bar females (BR—br).

	F2 Males.				F2 Females.			
	Red Bar.	White. Bar.	Red.	White.	Red Bar.	White Bar.	Red.	White.
(27.1)	50	37	32	50	49	31	42	34
".2	26	17	30	31	38	25	27	40
".3	39	32	29	42	40	21	33	30
".4	26	15	27	17	28	12	29	17
".5	53	26	55	41	51	27	35	29
".6	32	11	23	26	43	17	27	30
	226 (BR)	138 (Br)	196 (Rb)	207 (br)	249 (BR br)	133 (Br br)	193 (Rb br)	180 (br br)

As white bar-eyed flies and red-eyed flies are the cross-over classes, then the percentage of crossing-over is equal to 660, divided by 1,522, and the quotient, multiplied by 100, giving 43.3 per cent.

I next extracted pure lines of white bar-eyed flies and red-eyed flies, using the white bar-eyed males and red round-eyed females in the matings. Their factorial composition being (Br) for the males and (Rb—Rb) for the females.

F1 99 red-eyed males (Rb) and 105 red bar-eyed females (Rb—Br) F2 (from about thirty matings from F1).

Males.				Females.	
Red Bar.	White Bar.	Red.	White.	Red Bar.	Red.
1127 (BR)	1265 (Br)	1456 (Rb)	1045 (br)	2658 (BR or else Rb) (Br Rb)	2606 (Rb or else Rb) (br Rb)

The cross-over classes are the red-bar and white-eyed male flies. The percentage of crossing-over being 2,172, divided by 4,893, and the quotient, multiplied by 100, which gives 44.4 per cent. The difference, then, in the cross-over values when the linked factors entered in different ways was but 1.1 per cent., which does not seem to be a signicant difference.

J. D. IVES.

ON COUNTING CHROMOSOMES IN POLLEN-MOTHER CELLS

THE genetic study of hybrids between species with different chromosome numbers and of certain mutants requires the counting of many chromosome groups and raises the question of the best technique for the purpose. The staining qualities of aceto-carmine, which has long been used for preliminary work, especially by zoologists, are considerably improved by a trace of ferric salt. (Bolles Lee, in his well-known manual, gives formulæ for iron carmine; but this has no advantage for sections over iron hæmatoxylin.)

Iron Aceto-carmine 1.—Ordinary aceto-carmine is prepared by heating a 45 per cent. solution of glacial acetic acid to boiling with excess of powdered carmine, cooling and filtering. The young anthers are teased out with steel blades or needles in a drop of this until it changes slightly toward bluish red. An excess of iron spoils the preparation. Anther remains are removed, and a large thin coverglass (22 by 50 mm.) applied, using the minimum of liquid. The edges are sealed with vaseline. The preparation, if there is no excess of iron, may improve for a day or two.

Iron Aceto-carmine 2.—To a quantity of aceto-carmine a trace of a solution of ferric hydrate dissolved in 45 per cent. acetic acid is added until the liquid becomes bluish red, but no visible precipitate forms. An equal amount of ordinary aceto-carmine is then added. The anthers are teased out with nickel instruments. If the stain is too dark, more aceto-carmine is to be supplied. It may be diluted with 45 per cent. acetic.

Iron Aceto-carmine 3.—Anthers at the right stage are put into a mixture of 1 part of glacial acetic acid to 9 parts of absolute alcohol, to which sufficient solution of ferric hydrate in 45 per cent. acetic has been added to color the liquid brown (the amount

varies with different objects). After some days or weeks the anthers are teased out in ordinary aceto-carmine, avoiding the use of steel instruments.

The chromosomes are usually most accurately counted in the metaphase of the second division, in dicotyledons. When the preparation is a day or two old, the cytoplasm has swollen; and a slight tap on the thin coverglass above any particular cell will usually free the cytoplasm from the cell wall, and another tap flatten it out with its contained chromosomes.

Satisfactory results have been obtained by these methods during the past year with *Datura, Canna, Antirrhinum, Linaria, Brassica, Dahlia, Secale, Asparagus, Matthiola, Phaseolus, Stizolobium, Tradescantia, Hemerocallis, Iris, Gladiolus, Zea* and *Portulaca*. The methods failed with *Œnothera* and *Rhododendron*.

The second of the above methods will probably be of the widest applicability. The preparations will keep for a week or more, if an excess of stain and of iron are avoided. The method is quicker for counting chromosomes than staining sections with iron hæmatoxylin, and in favorable cases the results may be more certain. Thus in good preparations of *Datura* over a thousand pollen-mother cells are scattered singly on one slide, many of them showing the metaphase of the second division, and some having both plates in one plane, with the chromosomes well spaced and stained a deep bluish red, while the cytoplasm is unstained. It takes certainly a modicum of patience to acquire skill with this, as with most microscopical methods.

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